



08-21-00

A

Practitioner's Docket No. 65446-0053

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



Box Patent Application  
Assistant Commissioner for Patents  
Washington, D.C. 20231

NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of  
Inventor(s): **John S. Fox**

**WARNING:** 37 C.F.R. § 1.41(a)(1) points out:

*"(a) A patent is applied for in the name or names of the actual inventor or inventors.*

*(1) The inventorship of a nonprovisional application is that inventorship set forth in the oath or declaration as prescribed by § 1.63, except as provided for in § 1.53(d)(4) and § 1.63(d). If an oath or declaration as prescribed by § 1.63 is not filed during the pendency of a nonprovisional application, the inventorship is that inventorship set forth in the application papers filed pursuant to § 1.53(b), unless a petition under this paragraph accompanied by the fee set forth in § 1.17(i) is filed supplying or changing the name or names of the inventor or inventors."*

For (title): **HIGH SENSITIVITY BIOMOLECULE DETECTION  
WITH MAGNETIC PARTICLES**

CERTIFICATION UNDER 37 C.F.R. 1.10\*

*(Express Mail label number is mandatory.)*

*(Express Mail certification is optional.)*

I hereby certify that this correspondence and the documents referred to as attached therein are being deposited with the United States Postal Service on this date **August 18, 2000**, in an envelope as "Express Mail Post Office to Addressee," mailing Label Number **EL686767685US**, addressed to the: Box Patent Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

Wendy Balabon

*(type or print name of person mailing paper)*

Wendy Balabon

Signature of person mailing paper

**WARNING:** Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.

**\*WARNING:** Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. 1.10(b).  
"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.



## 1. Type of Application

This new application is for a(n)

(check one applicable item below)

- ☒ Original (nonprovisional)  
☐ Design  
☐ Plant

**WARNING:** Do not use this transmittal for a completion in the U.S. of an International Application under 35 U.S.C. 371(c)(4), unless the International Application is being filed as a divisional, continuation or continuation-in-part application.

**WARNING:** Do not use this transmittal for the filing of a provisional application.

**NOTE:** If one of the following 3 items apply, then complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF A PRIOR U.S. APPLICATION CLAIMED and a NOTIFICATION IN PARENT APPLICATION OF THE FILING OF THIS CONTINUATION APPLICATION.

- ☐ Divisional.  
☐ Continuation.  
☐ Continuation-in-part (C-I-P).

## 2. Benefit of Prior U.S. Application(s) (35 U.S.C. 119(e), 120, or 121)

**NOTE:** A nonprovisional application may claim an invention disclosed in one or more prior filed copending nonprovisional applications or copending international applications designating the United States of America. In order for a nonprovisional application to claim the benefit of a prior filed copending nonprovisional application or copending international application designating the United States of America, each prior application must name as an inventor at least one inventor named in the later filed nonprovisional application and disclose the named inventor's invention claimed in at least one claim of the later filed nonprovisional application in the manner provided by the first paragraph of 35 U.S.C. 112. Each prior application must also be:

- (i) An international application entitled to a filing date in accordance with PCT Article 11 and designating the United States of America; or
- (ii) Complete as set forth in § 1.51(b); or
- (iii) Entitled to a filing date as set forth in § 1.53(b) or § 1.53(d) and include the basic filing fee set forth in § 1.16; or
- (iv) Entitled to a filing date as set forth in § 1.53(b) and have paid therein the processing and retention fee set forth in § 1.21(l) within the time period set forth in § 1.53(f).

37 C.F.R. § 1.78(a)(1).

**NOTE:** If the new application being transmitted is a divisional, continuation or a continuation-in-part of a parent case, or where the parent case is an International Application which designated the U.S., or benefit of a prior provisional application is claimed, then check the following item and complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

**WARNING:** If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.

**WARNING:** When the last day of pendency of a provisional application falls on a Saturday, Sunday, or Federal holiday within the District of Columbia, any nonprovisional application claiming benefit of the provisional application must be filed prior to the Saturday, Sunday, or Federal holiday within the District of Columbia. See 37 C.F.R. § 1.78(a)(3).

☒ The new application being transmitted claims the benefit of prior U.S. application(s).  
Enclosed are ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE  
BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

3. **Papers Enclosed**

A. **Required for Filing Date under 37 C.F.R. § 1.53(b) (Regular) or 37 C.F.R. § 1.153 (Design) Application**

41 Pages of Specification

17 Pages of Claims

4 Sheets of Drawing

☐

Formal

☒

Informal

**WARNING:** *DO NOT* submit original drawings. A high quality copy of the drawings should be supplied when filing a patent application. The drawings that are submitted to the Office must be on strong, white, smooth, and non-shiny paper and meet the standards according to § 1.84. If corrections to the drawings are necessary, they should be made to the original drawing and a high-quality copy of the corrected original drawing then submitted to the Office. Only one copy is required or desired. For comments on proposed then-new 37 C.F.R. 1.84, see Notice of March 9, 1988. (1990 O.G. 57-62).

**NOTE:** "Identifying indicia, if provided, should include the application number or the title of the invention, inventor's name, docket number (if any), and the name and telephone number of a person to call if the Office is unable to match the drawings to the proper application. This information should be placed on the back of each sheet of drawing a minimum distance of 1.5 cm. (5/8 inch) down from the top of the page. . ." 37 C.F.R. § 1.84(c)).

(complete the following, if applicable)

☐

The enclosed drawing(s) are photograph(s), and there is also attached a "PETITION TO ACCEPT PHOTOGRAPH(S) AS DRAWING(S)." 37 C.F.R. § 1.84(b).

B. **Other Papers Enclosed**

2 Pages of declaration and power of attorney

1 Pages of Abstract

\_\_\_\_\_ Other

4. **Additional Papers Enclosed**

- ☐ Amendment to claims
- ☐ Cancel in this applications claims \_\_\_\_\_ before calculating the filing fee.  
(At least one original independent claim must be retained for filing purposes.)
- ☐ Add the claims shown on the attached amendment. (Claims added have been numbered consecutively following the highest numbered original claims.)
- ☐ Preliminary Amendment
- ☐ Information Disclosure Statement (37 C.F.R. § 1.98)
- ☐ Form PTO-1449 (PTO/SB/08A and 08B)
- ☐ Citations
- ☐ Declaration of Biological Deposit
- ☐ Submission of "Sequence Listing," computer readable copy and/or amendment pertaining thereto for biotechnology invention containing nucleotide and/or amino acid sequence.
- ☐ Authorization of Attorney(s) to Accept and Follow Instructions from Representative
- ☐ Special Comments
- ☐ Other

5. **Declaration or Oath (including power of attorney)**

**NOTE:** *A newly executed declaration is not required in a continuation or divisional application provided the prior nonprovisional application contained a declaration as required, the application being filed is by all or fewer than all the inventors named in the prior application, there is no new matter in the application being filed, and a copy of the executed declaration filed in the prior application (showing the signature or an indication thereon that it was signed) is submitted. The copy must be accompanied by a statement requesting deletion of the names of person(s) who are not inventors of the application being filed. If the declaration in the prior application was filed under § 1.47 then a copy of that declaration must be filed accompanied by a copy of the decision granting § 1.47 status or, if a nonsigning person under § 1.47 has subsequently joined in a prior application, then a copy of the subsequently executed declaration must be filed. See 37 C.F.R. § 1.63(d)(1)-(3).*

**NOTE:** *A declaration filed to complete an application must be executed, identify the specification to which it is directed, identify each inventor by full name, including the family name, and at least one given name without abbreviation together with any other given name or initial, and the residence, post office address and country of citizenship of each inventor, and state whether the inventor is a sole or joint inventor. 37 C.F.R. § 1.63(a)(1)-(4).*

- ☒ Enclosed  
Executed by

(check all applicable boxes)

- ☒ inventor(s).
- ☐ legal representative of inventor(s). 37 C.F.R. § 1.42 or 1.43.
- ☐ joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached.
- ☐ This is the petition required by 37 C.F.R. § 1.47 and the statement required by 37 C.F.R. § 1.47 is also attached. See item 13 below for fee.

- ☐ Not Enclosed.

**NOTE:** *Where the filing is a completion in the U.S. of an International Application, or where the completion of the U.S. application contains subject matter in addition to the International Application, the application may be treated as a continuation or continuation-in-part, as the case may be, utilizing ADDED PAGE FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION CLAIMED.*

- ☐ Application is made by a person authorized under 37 C.F.R. 1.41(c) on behalf of all the above named inventor(s).

*(The declaration or oath, along with the surcharge required by 37 C.F.R. § 1.16(e), can be filed subsequently).*

- ☐ Showing that the filing is authorized.  
*(not required unless called into question. 37 C.F.R. § 1.41(d))*

## 6. Inventorship Statement

**WARNING:** *If the named inventors are each not the inventors of all the claims an explanation, including the ownership of the various claims at the time the last claimed invention was made, should be submitted.*

The inventorship for all the claims in this application are:

- ☐ The same.

or

- ☐ Not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made,  
☐ is submitted.  
☐ will be submitted.

## 7. Language

**NOTE:** *An application including a signed oath or declaration may be filed in a language other than English. An English translation of the non-English language application and the processing fee of \$130.00 required by 37 C.F.R. § 1.17(k) is required to be filed with the application, or within such time as may be set by the Office. 37 C.F.R. § 1.52(d).*

- ☒ English  
☐ Non-English  
☐ The attached translation includes a statement that the translation is accurate.  
37 C.F.R. § 1.52(d).

## 8. Assignment

- ☐ An assignment of the invention to  
☐ is attached. A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or [ ]  
FORM PTO 1595 is also attached.  
☐ will follow.

**NOTE:** *"If an assignment is submitted with a new application, send two separate letters-one for the application and one for the assignment" Notice of May 4, 1990 (1114 O.G. 77-78).*

**WARNING:** *A newly executed "STATEMENT UNDER 37 C.F.R. § 3.73(b)" must be filed when a continuation-in-part application is filed by an assignee. Notice of April 30, 1993, 1150 O.G. 62-64.*

9. **Certified Copy**

Certified copy(ies) of application(s)

country	appln. no.	filed
country	appln. no.	filed
country	appln. no.	filed

from which priority is claimed

- ☐ is (are) attached.  
☐ will follow.

NOTE: The foreign application forming the basis for the claim for priority must be referred to in the oath or declaration. 37 C.F.R. § 1.55(a) and 1.63.

NOTE: This item is for any foreign priority for which the application being filed directly relates. If any parent U.S. application or International Application from which this application claims benefit under 35 U.S.C. 120 is itself entitled to priority from a prior foreign application, then complete item 18 on the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

10. **Fee Calculation (37 C.F.R. § 1.16)**

A. ☒ Regular application

CLAIMS AS FILED			
Number Filed	Number Extra	Rate	Basic Fee 37 CFR 1.16(a) \$ 690.00
Total Claims (37 CFR 1.16(c))	102 - 20 = 82	X \$ 18.00	1,476.00
Independent Claims (37 CFR 1.16(b))	20 - 3 = 17	X \$ 78.00	1,326.00
Multiple dependent claims, if any, (37 CFR 1.16(d))		X \$ 260.00	0

- ☐ Amendment cancelling extra claims is enclosed.  
☐ Amendment deleting multiple-dependencies is enclosed.  
☐ Fee for extra claims is not being paid at this time.

NOTE: If the fees for extra claims are not paid on filing they must be paid or the claims cancelled by amendment, prior to the expiration of the time period set for response by the Patent and Trademark Office in any notice of fee deficiency. 37 C.F.R. § 1.16(d)

Filing Fee Calculation \$ 3,492.00

B. ☐ Design application  
(\$310.00—37 C.F.R. § 1.16(f)) Filing Fee Calculation \$ \_\_\_\_\_

C. ☐ Plant application  
(\$480.00—37 C.F.R. § 1.16(g)) Filing Fee Calculation \$ \_\_\_\_\_

11. **Small Entity Statement(s)**

☒ Statement(s) that this is a filing by a small entity under 37 C.F.R. §§ 1.9 and 1.27 is (are) attached.

**WARNING:** "Status as a small entity must be specifically established in each application or patent in which the status is available and desired. Status as a small entity in one application or patent does not affect any other application or patent, including applications or patents which are directly or indirectly dependent upon the application or patent in which the status has been established. The refiling of an application under § 1.53 as a continuation, division, or continuation-in-part (including a continued prosecution application under § 1.53(d)), or the filing of a reissue application requires a new determination as to continued entitlement to small entity status for the continuing or reissue application. A nonprovisional application claiming benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) of a prior application, or a reissue application may rely on a statement filed in the prior application or in the patent if the nonprovisional application or the reissue application includes a reference to the statement in the prior application or in the patent and status as a small entity is still proper and desired. The payment of the small entity basic statutory filing fee will be treated as such a reference for purposes of this section." 37 C.F.R. § 1.28(a)(2).

(complete the following, if applicable)

☐ Status as a small entity was claimed in prior application \_\_\_\_\_, filed on \_\_\_\_\_ from which benefit is being claimed for this application under:

35 U.S.C. § ☐ 119(e),  
☐ 120,  
☐ 121,  
☐ 365(c),

and which status as a small entity is still proper and desired.

☐ A copy of the statement in the prior application is included.

Filing Fee Calculation (50% of A, B or C above) \$ 1,746.00

**NOTE:** Any excess of the full fee paid will be refunded if a small entity status is established refund request are filed within 2 months of the date of timely payment of a full fee. The two-month period is not extendable under § 1.136. 37 C.F.R. § 1.28(a)

12. **Request for International-Type Search** (37 C.F.R. § 1.104(d))

(complete, if applicable)

☐ Please prepare an international-type search report for this application at the time when national examination on the merits takes place.

13. Fee Payment Being Made at This Time

☐ Not Enclosed

☐ No filing fee is to be paid at this time.  
(This and the surcharge required by 37 C.F.R. § 1.16(e) can be paid subsequently.)

☒ Enclosed

☐ Filing fee \$ 1,746.00

☐ Recording assignment  
(\$40.00; 37 C.F.R. § 1.21(h))  
(See attached "COVER SHEET FOR  
ASSIGNMENT ACCOMPANYING NEW  
APPLICATION.") \$ \_\_\_\_\_

☐ Petition fee for filing by other  
than all the inventors or person  
on behalf of the inventor where  
inventor refused to sign or cannot  
be reached  
(\$130.00; 37 C.F.R. §§ 1.47 and 1.17(i)) \$ \_\_\_\_\_

☐ For processing an application with a  
specification in a non-English language  
(\$130.00; 37 C.F.R. §§ 1.52(d) and 1.17(k)) \$ \_\_\_\_\_

☐ Processing and retention fee  
(\$130.00; 37 C.F.R. §§ 1.53(d) and 1.21(l)) \$ \_\_\_\_\_

☐ Fee for international-type search report  
(\$40.00; 37 C.F.R. § 1.21(e)) \$ \_\_\_\_\_

NOTE: 37 C.F.R. § 1.21(l) establishes a fee for processing and retaining any application that is abandoned for failing to complete the application pursuant to 37 C.F.R. § 1.53(f) and this, as well as the changes to 37 C.F.R. § 1.53 and 1.78(a)(1), indicate that in order to obtain the benefit of a prior U.S. application, either the basic filing fee must be paid, or the processing and retention fee of § 1.21(l) must be paid, within 1 year from notification under § 53(f).

Total Fees Enclosed \$ 1,746.00

14. Method of Payment of Fees

☐ Check in the amount of \$ \_\_\_\_\_.

☒ Charge Account No. **18-0013** in the amount of \$ 1,746.00.  
A duplicate of this transmittal is attached.

NOTE: Fees should be itemized in such a manner that it is clear for which purpose the fees are paid. 37 C.F.R. § 1.22(b).



## 15. Authorization to Charge Additional Fees

**WARNING:** If no fees are to be paid on filing, the following items should not be completed.

**WARNING:** Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges, if extra claim charges are authorized.

☒ The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Account No. **18-0013**.

- ☒ 37 C.F.R. § 1.16(a), (f) or (g) (filing fees)  
☒ 37 C.F.R. § 1.16(b), (c) and (d) (presentation of extra claims)

**NOTE:** Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.16(d)), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action.

- ☒ 37 C.F.R. § 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)  
☒ 37 C.F.R. § 1.17(a)(1)-(5) (extension fees pursuant to § 1.136(a).  
☒ 37 C.F.R. § 1.17 (application processing fees)

**NOTE:** "A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).

☐ 37 C.F.R. § 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. § 1.311(b))

**NOTE:** Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b)).

**NOTE:** 37 C.F.R. § 1.28(b) requires "Notification of any change in status resulting in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying, . . . issue fee." From the wording of 37 C.F.R. § 1.28(b), (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

16. Instructions as to Overpayment

NOTE: "... Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

☒ Credit Account No. 18-0013.


☐ Refund.

Date: 8/18/00

Reg. No. 41,882

Tel. No.: (248) 594-0656

Customer No. 010291

  
**SIGNATURE OF PRACTITIONER**  
James F. Kamp, Esq.  
RADER, FISHMAN & GRAUER PLLC  
39533 North Woodward Avenue,  
Suite 140  
Bloomfield Hills, Michigan 48304

☒ Incorporation by reference of added pages

(check the following item if the application in this transmittal claims the benefit of prior U.S. application(s) (including an international application entering the U.S. stage as a continuation, divisional or C-I-P application) and complete and attach the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED)

☒ Plus Added Pages for New Application Transmittal Where Benefit of Prior U.S. Application(s) Claimed

Number of pages added \_\_\_\_\_

☐ Plus Added Pages for Papers Referred to in Item 4 Above

Number of pages added \_\_\_\_\_

☐ Plus added pages deleting names of inventor(s) named on prior application(s) who is/are no longer inventor(s) of the subject matter claimed in this application.

Number of pages added \_\_\_\_\_

☐ Plus "Assignment Cover Letter Accompanying New Application"

Number of pages added \_\_\_\_\_

☐ Statement Where No Further Pages Added

(if no further pages form a part of this Transmittal, then end this Transmittal with this page and check the following item)

☐ This transmittal ends with this page.

r0056189

**ADDED PAGES FOR APPLICATION TRANSMITTAL WHERE BENEFIT OF  
PRIOR U.S. APPLICATION(S) CLAIMED**

NOTE: See 37 C.F.R. § 1.78.

**17. Relate Back**

**WARNING:** If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.

(complete the following, if applicable)

☐ Amend the specification by inserting, before the first line, the following sentence:

**A. 35 U.S.C. 119(e)**

NOTE: "Any nonprovisional application claiming the benefit of one or more prior filed copending provisional applications must contain or be amended to contain in the first sentence of the specification following the title a reference to each such prior provisional application, identifying it as a provisional application, and including the provisional application number (consisting of series code and serial number)." 37 C.F.R. § 1.78(a)(4).

☒ "This application claims the benefit of U.S. Provisional Application(s) No(s).:

**APPLICATION NO(S):****60/150210****FILING DATE****August 21, 1999**

**B. 35 U.S.C. 120, 121 and 365(c)**

NOTE: "Except for a continued prosecution application filed under § 1.53(d), any nonprovisional application claiming the benefit of one or more prior filed copending nonprovisional applications or international applications designating the United States of America must contain or be amended to contain in the first sentence of the specification following the title a reference to each such prior application, identifying it by application number (consisting of the series code and serial number) or international application number and international filing date and indicating the relationship of the applications. . . . Cross-references to other related applications may be made when appropriate." (See § 1.14(a)). 37 C.F.R. § 1.78(a)(2)

☐ "This application is a

☐ continuation

☐ continuation-in-part

☐ divisional

of copending application(s)

☐ application number \_\_\_\_\_ filed on \_\_\_\_\_ "

☐ International Application \_\_\_\_\_ filed on \_\_\_\_\_ and which designated the U.S."

NOTE: The proper reference to a prior filed PCT application that entered the U.S. national phase is the U.S. serial number and the filing date of the PCT application that designated the U.S.

NOTE: (1) Where the application being transmitted adds subject matter to the International Application, then the filing can be as a continuation-in-part or (2) if it is desired to do so for other reasons then the filing can be as a continuation.

NOTE: The deadline for entering the national phase in the U.S. for an international application was clarified in the Notice of April 28, 1987 (1079 O.G. 32 to 46) as follows:

"The Patent and Trademark Office considers the International application to be pending until the 22nd month from the priority date if the United States has been designated and no Demand for International Preliminary Examination has been filed prior to the expiration of the 19th month from the priority date and until the 32nd month from the priority date if a Demand for International Preliminary Examination which elected the United States of America has been filed prior to the expiration of the 19th month from the priority date, provided that a copy of the international application has been communicated to the Patent and Trademark Office within the 20 or 30 month period respectively. If a copy of the international application has not been communicated to the Patent and Trademark Office within the 20 or 30 month period respectively, the international application becomes abandoned as to the United States 20 or 30 months from the priority date respectively. These periods have been placed in the rules as paragraph (h) of § 1.494 and paragraph (i) of § 1.495. A continuing application under 35 U.S.C. 365(c) and 120 may be filed anytime during the pendency of the international application."

☐ "The nonprovisional application designated above, namely application \_\_\_\_\_, filed \_\_\_\_\_, claims the benefit of U.S. Provisional Application(s) No(s).:

**APPLICATION NO(S).:**

**FILING DATE**

☐ Where more than one reference is made above please combine all references into one sentence.

18. Relate Back—35 U.S.C. 119 Priority Claim for Prior Application

The prior U.S. application(s), including any prior International Application designating the U.S., identified above in item 17B, in turn itself claim(s) foreign priority(ies) as follows:

Country	Appln. no.	Filed
---------	------------	-------

The certified copy(ies) has (have)

☐ been filed on \_\_\_\_\_, in prior application \_\_\_\_\_, which was filed on \_\_\_\_\_.

☐ is (are) attached.

**WARNING:** The certified copy of the priority application that may have been communicated to the PTO by the International Bureau may not be relied on without any need to file a certified copy of the priority application in the continuing application. This is so because the certified copy of the priority application communicated by the International Bureau is placed in a folder and is not assigned a U.S. serial number unless the national stage is entered. Such folders are disposed of if the national stage is not entered. Therefore, such certified copies may not be available if needed later in the prosecution of a continuing application. An alternative would be to physically remove the priority documents from the folders and transfer them to the continuing application. The resources required to request transfer, retrieve the folders, make suitable record notations, transfer the certified copies, enter and make a record of such copies in the Continuing Application are substantial. Accordingly, the priority documents in folders of international applications that have not entered the national stage may not be relied on. Notice of April 28, 1987 (1079 O.G. 32 to 46).

19. Maintenance of Copendency of Prior Application

**NOTE:** The PTO finds it useful if a copy of the petition filed in the prior application extending the term for response is filed with the papers constituting the filing of the continuation application. Notice of November 5, 1985 (1060 O.G. 27).

A. ☐ Extension of time in prior application

(This item **must** be completed and the papers filed **in the prior application**, if the period set in the prior application has run.)

☐ A petition, fee and response extends the term in the pending **prior** application until

☐ A **copy** of the petition filed in prior application is attached.

B. ☐ Conditional Petition for Extension of Time in Prior Application

(complete this item, if previous item not applicable)

☐ A conditional petition for extension of time is being filed in the pending **prior** application.

☐ A **copy** of the conditional petition filed in the prior application is attached.

**20. Further Inventorship Statement Where Benefit of Prior Application(s) Claimed**

*(complete applicable item (a), (b) and/or (c) below)*

- (a) ☐ This application discloses and claims only subject matter disclosed in the prior application whose particulars are set out above and the inventor(s) in this application are

☐ the same.

☐ less than those named in the prior application. It is requested that the following inventor(s) identified for the prior application be deleted:

---

*(type name(s) of inventor(s) to be deleted)*

- (b) ☐ This application discloses and claims additional disclosure by amendment and a new declaration or oath is being filed. With respect to the prior application, the inventor(s) in this application are

☐ the same.

☐ the following additional inventor(s) have been added:

---

*(type name(s) of inventor(s) to be deleted)*

- (c) ☐ The inventorship for all the claims in this application are

☐ the same.

☐ not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made

☐ is submitted.

☐ will be submitted.

**21. Abandonment of Prior Application (if applicable)**

- ☐ Please abandon the prior application at a time while the prior application is pending, or when the petition for extension of time or to revive in that application is granted, and when this application is granted a filing date, so as to make this application copending with said prior application.

**NOTE:** According to the Notice of May 13, 1983 (103, TMOG 6-7), the filing of a continuation or continuation-in-part application is a proper response with respect to a petition for extension of time or a petition to revive and should include the express abandonment of the prior application conditioned upon the granting of the petition and the granting of a filing date to the continuing application.

## 22. Petition for Suspension of Prosecution for the Time Necessary to File an Amendment

**WARNING:** "The claims of a new application may be finally rejected in the first Office action in those situations where (1) the new application is a continuing application of, or a substitute for, an earlier application, and (2) all the claims of the new application (a) are drawn to the same invention claimed in the earlier application, and (b) would have been properly finally rejected on the grounds of art of record in the next Office action if they had been entered in the earlier application." MPEP, § 706.07(b), 6th ed., rev.2. ☐

**NOTE:** Where it is possible that the claims on file will give rise to a first action final for this continuation application and for some reason an amendment cannot be filed promptly (e.g., experimental data is being gathered) it may be desirable to file a petition for suspension of prosecution for the time necessary.

(check the next item, if applicable)

- ☐ There is provided herewith a Petition To Suspend Prosecution for the Time Necessary to File An Amendment (New Application Filed Concurrently)

## 23. Small Entity (37 CFR § 1.28(a))

- ☐ Applicant has established small entity status by the filing of a statement in parent application \_\_\_\_\_ on \_\_\_\_\_.  
☐ A copy of the statement previously filed is included.

**WARNING:** See 37 CFR § 1.28(a).

## 24. NOTIFICATION IN PARENT APPLICATION OF THIS FILING

- ☐ A notification of the filing of this  
(check one of the following)
- ☐ continuation
  - ☐ continuation-in-part
  - ☐ divisional

is being filed in the parent application, from which this application claims priority under 35 U.S.C. § 120.

R0056201

Practitioner's Docket No. 65446-0053

PATENT

In re application of: John S. Fox  
 Application No.: To Be Assigned  
 Filed on: Herewith

Title: **HIGH SENSITIVITY BIOMOLECULE DETECTION WITH MAGNETIC PARTICLES**

**STATEMENT CLAIMING SMALL ENTITY STATUS  
 (37 CFR 1.9(f) and 1.27(b)--INDEPENDENT INVENTOR)**

As a below named inventor, I hereby state that I qualify as an independent inventor, as defined in 37 CFR 1.9(c), for purposes of paying reduced fees to the United States Patent and Trademark Office under Sections 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office, with regard to the invention described in

- ☒ the specification filed herewith, with title as listed above.  
☐ the application identified above.  
☐ the patent identified above.

I have not assigned, granted, conveyed or licensed, and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who would not qualify as an independent inventor under 37 CFR 1.9(c), if that person had made the invention, or to any concern that would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

- ☒ No such person, concern, or organization exists.  
☐ Each such person, concern or organization is listed below.

*\*NOTE: Separate statements are required from each named person, concern or organization having rights to the invention as to their status as small entities. (37 CFR 1.27)*

FULL NAME \_\_\_\_\_

ADDRESS \_\_\_\_\_

\_\_\_\_\_  
☐ INDIVIDUAL    ☐ SMALL BUSINESS CONCERN    ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

*(check the following item, if desired)*

142



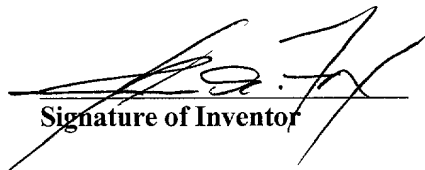
## Practitioner's Docket No. 65446-0053

NOTE The following verification statement need not be made in accordance with the rules published on October 10, 1997, 62 Fed Reg 52131, effective December 1, 1997

NOTE. "The presentation to the Office (whether by signing, filing, submitting, or later advocating) of any paper by a party, whether a practitioner or non-practitioner, constitutes a certification under § 10.18(b) of this chapter. Violations of § 10.18(b)(2) of this chapter by a party, whether a practitioner or non-practitioner, may result in the imposition of sanctions under § 10.18(c) of this chapter. Any practitioner violating § 10.18(b) may also be subject to disciplinary action. See §§ 10.18(d) and 10.23(c)(15)." 37 CFR 1.4(d)(2).

☒ I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

John S. Fox

  
Signature of Inventor

Date August 15, 2000

R0090956

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**HIGH SENSITIVITY BIOMOLECULE DETECTION  
WITH MAGNETIC PARTICLES**5                    **CROSS-REFERENCE TO RELATED APPLICATION**

This application claims priority based on U.S. provisional patent application no. 60/150210 filed August 21, 1999.

**FIELD OF THE INVENTION**

10            The present invention generally relates to the field of biomolecule detection. More specifically, the present invention relates to compositions, methods, and systems for the detection and manipulation of biomolecules using magnetic particles.

**BACKGROUND OF THE INVENTION**

15            In basic research, one goal is to understand how genes are distributed within populations and how expression of those genes leads to phenotypic differences. This information has the potential to become a powerful tool for predicting human health trends and has been a driving force behind the search for genetic markers for human disease.

20            Over the years, many biochemical techniques have been introduced for analyzing the presence and/or amount of a biomolecule in a sample. As examples, a number of organic stains have been adapted for the detection of electrophoretically separated proteins, including Bromphenol Blue, Coomassie Blue, Fast Green (Food Green 3) and Amido Black (Acid Black 1). (See Durrem, *J. Am. Chem. Soc.* 72:2943 (1950), Grassman and Hannig, *Z. Physiol. Chem.* 290:1 (1952), Fazekas De St. Groth et al., *Biochim. Biophys. Acta* 71:377 (1963), and Meyer and Lamberts, *Biochim. Biophys. Acta* 107:144 (1965)). Fluorescent stains, such as fluorescamine and 2-methoxy-2,4-diphenyl-3(2H)-Furanone (MDPH), are also used to detect proteins (See Ragland et al., *Anal. Biochem.* 59:24 (1974) and Pace et al., *Biochem. Biophys. Res. Commun.* 57:482 (1974)). A sensitive technique for staining proteins is silver staining. (See Merrill et al., *Proc. Natl. Acad. Sci. USA* 76:4335 (1979) and Switzer et al., *Anal. Biochem.* 25            98:231 (1979)). While these techniques may be useful to resolve total protein in a 30

sample, they are limited in their usefulness to detect a specific protein in a heterogeneous population of proteins.

The detection of specific proteins in a sample can be accomplished by techniques including Western blot, immunoprecipitation, enzyme-linked immunoassay (ELISA), and sandwich assays. These techniques typically use radioactivity, fluorescence, and chemiluminescence to label or mark an antibody or other protein which binds to the target protein and thereby identifies the presence and/or location of the target. Depending on the quality of the antibody and the label used, the sensitivity of detection and non-specific binding varies.

Radioactivity, fluorescence, and chemiluminescence are also commonly used for the detection of specific nucleic acid sequences in a sample. Hybridization techniques, such as Southern and Northern blotting, are frequently employed to detect the presence of polymorphisms in a nucleic acid sample. In nucleic acid hybridization, for example, a radioactive label (e.g.  $^{32}\text{P}$  or  $^{35}\text{S}$ ) is incorporated into an oligonucleotide probe which complements a target nucleic acid, and hybridization with the target is accomplished at a specific salt concentration and temperature. (See e.g. Sambrook, J. et al., *Molecular Cloning, A Laboratory Manual* (1989)).

Southern et al. has used nucleic acid hybridization by setting up an array of oligonucleotides on plastic and glass, probing with a radioactive oligonucleotide, and detecting the presence of a target nucleic acid with a PhosphorImager. (See Southern, E.M. et al., *Nucleic Acids Res* 22, 1368-1373 (1994)). The PhosphorImager instrument, an expensive laser based optical system, and clean image-ready phosphor screens are needed for each sample read, making the system both cumbersome and very expensive. In addition, radioactive probes have a short shelf life ( $T_2$  = days to months) and require tight inventory control in a licensed facility. Although some companies are currently performing genetic screening using this method, the cost is prohibitive for most diagnostic procedures.

Others in the field are pursuing methods more predisposed to automation in hopes of enabling the rapid screening of a sample for a number of sequences. As one example, Affymetrix (Santa Clara, CA) has described a system which performs on-chip hybridization. (See Kreiner, T., *American Laboratory* March:39-43 (1996). In this

system, oligonucleotides are arrayed in 90x90  $\mu\text{m}$  cells with  $10^7$  oligonucleotides per cell, with 20,000 probe cells on each chip. This is annealed with fluorescence-labeled probes, and detection is carried out using a 488 nm Argon laser (8  $\mu\text{m}$  spot size) as an excitation source and a photomultiplier tube to detect the fluorescence emission. To read the chip, an optical system consisting of a dichromic mirror, scanning head, routing mirror and a confocal optical system are employed. One significant problem with this approach is non-specific background. Several natural occurring molecules either contribute to or quench the fluorescent signal, making this technique prone to a background noise which prevents this system from achieving highly sensitive nucleic acid detection.

Chemiluminescence is another marker employed to detect biomolecules. Chemiluminescence uses an enzyme coupled to the probe which catabolizes a chemical substrate to generate a photon. (See Bronstein, et al., *BioTechniques* 8:310-313 (1990)). Chemiluminescent nucleic acid hybridization assays may use a high performance, low-light-sensitive charge coupled device (CCD) camera to image the light emission from the chemical reaction. Often the camera is controlled by a personal computer and the images are archived on diskettes. While the CCD cameras are robust, CCD based systems do not have the sensitivity of film and the reagents have a one-year shelf life when stored at 4°C (Tropix Inc.). As with fluorescence detection approaches, this approach is limited by background noise caused by naturally occurring enzymes or compounds contributing to the signal.

As the secrets of genomic regulation and the biosynthesis of enzymes, receptors, and ligands involved in human disease unfold, the need for detection techniques which provide a high degree of specificity and sensitivity with minimal background noise, while minimizing cost and handling issues, is manifest. In view of the foregoing, and notwithstanding the various efforts exemplified in the prior art, there remains a need for novel compositions, methods, and systems for highly sensitive biomolecule detection.

SUMMARY OF THE INVENTION

Recognizing the limitations associated with current techniques for detection, manipulation and separation of biomolecules, the present invention provides methods and systems for the detection and manipulation of biomolecules using magnetic particles. Through its embodiments, the present invention improves specificity and sensitivity while minimizing background, cost and handling issues related to biomolecule detection and manipulation.

The present invention includes highly sensitive biomolecule detection methods and systems which use a magnetic moiety as a marker to determine the presence and/or location of a specific target biomolecule. The invention detects target molecules or molecular biomolecules that have been contacted directly or indirectly with a magnetically labeled probe by subjecting the target-probe complex to an applied magnetic field and determining the resulting magnetic characteristics. The invention provides methods and systems to prepare such magnetic probes and to identify the presence and/or location of the target biomolecules disposed on a support or in solution. The invention may detect characteristic responses of samples by several means, including but not limited to, by induced magnetization or orientation changes of magnetically labeled biomolecules.

The present invention also provides methods and systems for nucleic acid hybridization using magnetic labels, ferrofluids, and nonmagnetic colloids, as some examples.

The present invention also provides methods and systems to study competitive binding and also provides methods and systems which use magnetic labels to screen for, manipulate, and separate target biomolecules, for example, in the same sample.

Methods and systems for the detection and separation of nucleic acids, as one example only, using ferrofluids and beads, are also provided. The invention also includes methods and systems to enhance the binding of a probe to a target biomolecule and methods to reduce the background noise in hybridizations and binding assays.

### BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 displays a block diagram of an embodiment of a magnetic detection apparatus used to detect a magnetic particle joined to a biomolecule.

FIGURE 2 is a graph of a hysteresis loop for a ferromagnetic material.

5        FIGURE 3 is a graph of a portion of the hysteresis loops for neodymium iron boron and samarium cobalt.

FIGURE 4 illustrates a typical output of an embodiment of a magnetic detection system in which a ferrofluid-labeled DNA sample was inserted into the reader at  $t=in$  and removed at  $t=out$ ; the smooth line represents a 200 point running average.

10        FIGURE 5 shows a semi-log plot of varying amounts of ferrofluid-labeled plasmid dsDNA (■) or an oligonucleotide (○) spotted on a support and detected with an embodiment of a magnetic detection system; the relative magnetic unit reading (RMU) was the maximum voltage deflection of the spot corrected for background voltage and the line represents the mathematical fit to the data (equation is in the inset).

15        FIGURE 6 shows a semi-log plot of varying amounts of ferrofluid-labeled RNA (■) spotted on a support and detected with an embodiment of a magnetic detection system; the relative magnetic unit reading (RMU) was the maximum voltage deflection of the spot corrected for background voltage and the line represents the mathematical fit to the data (equation is in the inset).

20        DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention comprises highly sensitive biomolecule detection methods and systems which use a magnetic moiety as a marker to determine the presence, location, and/or quantity of a specific target biomolecule, by way of examples only, a protein, lipid, or a nucleic acid, in a sample. Many types of magnetic labels may be joined to biomolecules in embodiments of the present invention. As examples only, preferred magnetic labels may include  $Fe^3O^4$ ,  $Fe^2O^3$ , and rare-earth elements with atomic numbers between 64 and 69, inclusive, which have been incorporated into a colloidal suspension. In some preferred embodiments the magnetic labels are attached directly to the biomolecule, as one example only, a ferrofluid bound to a nucleic acid or protein, or in other embodiments, the magnetic label is indirectly attached to the

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biomolecule, e.g., through an intermediate, such as an antibody, a binding protein (e.g., avidin, streptavidin, and derivatives thereof), or a chemical linker.

Although attachment of a magnetic label, such as a ferrofluid, to a biomolecule disposed on a support is used in some embodiments of the present invention for the rapid identification of the presence, location, or quantity of a biomolecule, magnetically labeled biomolecules are also used as magnetic probes to specifically identify a target biomolecule which may be present in a heterogeneous sample of biomolecules. Accordingly, the invention provides methods and systems to prepare such magnetic probes and to identify the presence and/or location of the target biomolecules disposed on a support. One skilled in the art will appreciate that conventional approaches to nucleic acid hybridization and protein identification (e.g., immunoblotting and ELISA) are readily adapted to the magnetic detection methods and systems disclosed in preferred embodiments of the present invention. Furthermore, the present invention provides methods and systems to study competitive binding and techniques which enable the screening for several target biomolecules in the same sample.

In one embodiment, the present invention detects magnetically labeled biomolecules by measuring or characterizing the magnetic signal generated by the magnetic particle joined to the biomolecule. In several preferred embodiments, the invention uses a sensitive magnetic sensor, such as a giant magnetoresistive ratio sensor (GMR), for the detection of magnetically labeled biomolecules. A method of biomolecule detection according to one preferred embodiment of the present invention is as follows: A biomolecule is disposed on a support. Then the biomolecule is bound with a magnetic label which generates a magnetic signal, or the biomolecule is bound with a magnetically-labeled probe which generates a magnetic signal. The unbound magnetic label or unbound magnetically-labeled probe is removed by washing. Subsequently, the support having the biomolecule and attached magnetic label or biomolecule-magnetic probe complex is passed before a magnetic sensor which detects the presence and location of the magnetic probes and their specific magnetic characteristics. By characterizing the properties of a magnetically labeled biomolecule in an applied magnetic field, as one example only, by defining the hysteresis loop, solving one or more of the parameters of the hysteresis loop (e.g., saturation

magnetization, remnant magnetization, and coercive force) or both, the identity as well as the quantity and location of the magnetic label are determined.

Additionally, the invention comprises methods and systems for the detection of one or more different biomolecules in the same sample by using magnetically labeled probes having different magnetic particles. Because many different magnetic particles exist and each has a unique magnetic signature, the detection of several different magnetically labeled biomolecules in the same sample is accomplished. Notably, the size and geometry of the magnetic particle affect magnetic characteristics, and therefore magnetic labels with homogeneous magnetic particles are preferred.

Further, the invention comprises methods and systems which permit the rapid screening for the presence of one or more target biomolecules in a solution. In one embodiment, one or more colored beads joined to a probe biomolecule, as examples only, an antibody or an oligonucleotide, are used to detect magnetically labeled target biomolecules in a solution. In additional embodiments, one or more different colored beads are joined to one or more different probe biomolecules so as to detect the presence and amount of several different target biomolecules in the same sample solution. Accordingly, the invention comprises methods by which colored beads having one or more probe biomolecules are bound to magnetically labeled target biomolecules, and a magnetic field is applied so as to separate colored bead-probe-target complexes from colored beads having probe biomolecules which have not bound to magnetically labeled target biomolecules. The presence and amount of target biomolecules in the sample is then determined by the efficiency at which the colored beads respond to the applied magnetic field, as detected visually or with the aid of electronic and/or optical devices, such as a spectrophotometer or a photodiode CCD camera.

Additional embodiments may include, as examples only, fluorescent beads or fluorescent cells which produce fluorescent signals (for example, like green fluorescent protein), with subsequent measurement of fluorescence.

In additional embodiments, the presence of a target biomolecule in a sample solution is determined by monitoring the time it takes for a magnetic particle to reorient itself in response to a repositioning of an applied magnetic field ("magnetic swing time"). Because magnetically labeled probes attached to target biomolecules, for



example, reorient their alignment to a shifted magnetic field more slowly than unattached magnetically labeled probes, the presence of a target biomolecule in a sample solution is easily determined. Preferably, embodiments that detect the presence of a target biomolecule in a sample solution by "magnetic swing time" have magnetically labeled probes attached to beads that exhibit a strong polarization orientation in a magnetic field or magnetically labeled probes that have magnetic particles that are needle-like.

The invention also comprises methods and systems to enhance the binding of a probe to a target biomolecule and methods to reduce the background noise in hybridizations and binding assays. By applying a magnetic or electric field, or both, to regions of a support where a target biomolecule is disposed, for example, the movement toward and concentration of a magnetically labeled probe biomolecule near the region of the support having the target biomolecule is obtained. Advantages include improved binding kinetics and conservation of probe materials. Alternatively, a magnetic or electrical field, or both, is applied after a target biomolecule is bound by the magnetically labeled probe biomolecule so as to remove or separate from the target biomolecule and support any unbound or non-specifically bound magnetically labeled probe biomolecules. Further, the invention provides methods and systems by which ferrofluid-labeled biomolecules are efficiently separated according to their magnetic potential, and in which ferrofluid-labeled biomolecules in a solution are separated in an applied magnetic field. Because the amount of ferrofluid bound to the biomolecule is directly related to the mass of the biomolecule, the invention comprises a magnetic-mass based separation technique in one embodiment.

Some preferred embodiments use types of magnetic labels. A "magnetic label" or "magnetic marker" is any transiently or permanently magnetized entity. In some embodiments of the present invention, a magnetic label comprises a magnetic particle that is ferromagnetic or ferrimagnetic or paramagnetic or superparamagnetic. The magnetic markers or labels preferably generate a magnetic signal, which can be, by way of example only, the magnetic field generated by ferromagnetic and ferrimagnetic materials, or the attraction for magnets characteristic of paramagnetic and

superparamagnetic materials. In solution, the magnetic moments of the particles desirably align with each other.

5 In some embodiments, a magnetic label comprises a plurality of colloidal iron particles that define a respective magnetic moment. The term "magnetic labels" also refers to magnetic particles which comprise metal, metal compounds, or nuclei coated with a metal or metal compound. In some embodiments, preferable magnetic labels include ferrofluids or other magnetizable colloids. Additionally, the term "magnetic label" refers to a magnetic particle including iron, cobalt, nickel, ferrous oxide, ferrous hydroxide, and other ferrous alloys, disposium oxide, and rare earth elements with  
10 atomic numbers between 64 and 69, inclusive, or magnetite ( $\text{Fe}_3\text{O}_4$ ), maghemite ( $\text{Fe}_2\text{O}_3$ ), and other mixed oxides. Magnetic labels having rare-earth magnetic particles are desirable because they may have a five-fold greater magnetization than iron oxide beads. The term "magnetic label" also refers to the magnets discussed in Vassiliou et al., *J. Appl. Physics* 73(10); 5109 (1993)), the disclosure of which is hereby incorporated by  
15 reference in its entirety.

One of ordinary skill in the art will appreciate that there are available biomolecule separation techniques that can be used prior to disposing a desired biomolecule on a support or used to separate and dispose the biomolecule on a support. There may be advantages for separating the desired biomolecule from other  
20 biomolecules present in a sample prior to contacting the sample with a magnetic label or a magnetically labeled probe biomolecule. Notably, the separation of the desired biomolecule often facilitates the isolation of the biomolecule after identification. The separation of the desired biomolecule from others in the sample is not necessary, however, to practice preferred embodiments of the present invention.

25 The present invention includes several methods and systems by which a target biomolecule can be disposed on a support in preparation for detection with magnetic labels or magnetically labeled probe biomolecules.

The separation of biomolecules prior to detection is accomplished, for example, by a one-dimensional or two-dimensional electrophoresis procedure. (See e.g., Methods in Enzymology Vol. 182, Guide to Protein Purification, ed. Deutscher, Academic Press  
30 Inc. pp. 425-477, San Diego, California (1990), Current Protocols in Molecular Biology,

Ausubel et al., ed., John Wiley & Sons (1994-1998), and Sambrook et al., Molecular Cloning: A Laboratory Manual, 2 ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1989)). Denaturing and non-denaturing gel electrophoresis are frequently used to separate nucleic acids, and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS/PAGE) is a common method to separate proteins. Further, pulse-field electrophoresis, two-dimensional protein electrophoresis, isoelectric focussing, and other separation techniques are used to separate target biomolecules prior to detection with a magnetic label or a magnetically labeled probe. Additionally, biomolecules can be separated chromatographically, for example, by thin layer chromatography (TLC), by liquid chromatography techniques, such as high performance liquid chromatography (HPLC) or fast performance liquid chromatography (FPLC), or by affinity chromatography techniques, prior to detection with a magnetic label or a magnetically labeled probe.

Another common laboratory technique called "blotting" is also used to dispose a target biomolecule on a support. This technique allows for the transfer of separated biomolecules on a matrix to a solid membrane or a filter. (See e.g., Current Protocols in Molecular Biology, Ausubel et al., ed., John Wiley & Sons (1994-1998), and Sambrook et al., Molecular Cloning: A Laboratory Manual, 2 ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1989)). Additionally, biomolecules disposed on a membrane support by blotting are frequently immobilized or fixed into position so that further rounds of detection can be accomplished. By "stripping" or removing the first bound probe by techniques known in the art, subsequent rounds of detection with new magnetically labeled probes are performed.

In preferred embodiments, the present invention may include a "matrix" or "support" which may be a carrier, a bead, a resin, or any macromolecular structure used to attach, join, immobilize, or dispose thereon a biomolecule, by way of examples only, a nucleic acid, lipid, or protein. Supports may include, but are not limited to, the walls of wells of a reaction tray, test tubes, polystyrene beads, fluorescent beads, magnetic beads, nitrocellulose strips, membranes, microparticles such as latex particles, sheep (or other animal) red blood cells, cells, fluorescent particles, duracytes® and others. Additionally, organic carriers including proteins and oligo/polysaccharides (e.g. cellulose,

starch, glycogen, chitosane or aminated sepharose) and inorganic carriers such as silicon oxide material (e.g. silica gel, zeolite, diatomaceous earth or aminated glass) may be used in embodiments of the present invention. Furthermore, in some embodiments, a liposome or lipid bilayer (natural or synthetic) may be used as a support. Desirable supports may also include polyacrylamide gels, agarose gels, composite gels, and other gel matrices, papers, chips, membranes, chromatography matrices, as used in thin layer chromatography, and resins or beads, as used in affinity chromatography.

In some embodiments of the present invention, the support has a hydrophobic surface that interacts with a portion of the biomolecule by hydrophobic non-covalent interaction. As one example only, the hydrophobic surface of the support is oftentimes a polymer such as plastic or any other polymer in which hydrophobic groups have been linked, such as polystyrene, polyethylene or polyvinyl. In some embodiments, the support has a charged surface which interacts with the biomolecule, as one example only, a charged nitrocellulose or nylon membrane. In other embodiments, the support is attached to a biomolecule through a linker, such as biotin-avidin or biotin-streptavidin, or biotin and an avidin or streptavidin derivative. The supports used in some embodiments of the present invention have other reactive groups which are chemically activated so as to attach a biomolecule. As some examples, cyanogen bromide activated matrices, epoxy activated matrices, thio and thiopropyl gels, nitrophenyl chloroformate and N-hydroxy succinimide chlorformate linkages, and oxirane acrylic supports are adapted for use in some embodiments.

In the present invention, any type of biomolecule, by way of examples only, proteins, polypeptides, nucleic acids, and lipids, can be joined or disposed on a support and subsequently joined to a magnetic label. Further, preparations of biological samples having biomolecules can be joined or disposed on a support. In preferred embodiments, several different biomolecules or different preparations of biological samples having biomolecules are attached to a support in an ordered array wherein each biomolecule or preparation of biological sample is attached to a distinct region of the support which does not overlap with the attachment site of any other biomolecule or preparation of biological sample. Preferably, such an ordered array is designed to be "addressable"

where the distinct locations are recorded and can be accessed as part of an assay procedure.

In some embodiments, addressable biomolecule arrays comprise a plurality of different biomolecule probes that are joined to a support in different known locations. The knowledge of the precise location of each biomolecule probe makes these "addressable" arrays particularly useful in binding assays. As one example only, an addressable array can comprise a support joined to many different antibodies that recognize different human proteins that are tumor markers for various forms of cancer. The proteins from a preparation of biological sample from a human subject are magnetically labeled (e.g., using a ferrofluid labeling process, discussed below), and the magnetically labeled sample is applied to the array under conditions that permit antibody binding. If a protein in the sample is recognized by an antibody on the array, then a magnetic signal will be detected at a position on the support that corresponds to the antibody-protein complex. Since each antibody and its position on the array are known, an identification of the protein/tumor marker and, thus, the disease state of the subject, are rapidly determined. Additionally, one embodiment can employ nucleic acid probes joined to a support to form an array of magnetically labeled nucleic acids from a biological sample from a human subject. In this manner, by way of example, disease prognosis may be assessed based on the use of nucleic acid probes which are associated with sequences that have been associated with human disease and the detection of magnetically labeled complementary nucleic acid sequences present in the biological sample. These approaches are easily automated using technology known to those of skill in the art of high throughput diagnostic analysis.

The present invention may comprise in its embodiments any addressable array technology known in the art. One embodiment of polynucleotide arrays is known as the Genechips™, and has been generally described in U.S. Patent No. 5,143,854; PCT publications WO 90/15070 and 92/10092. These arrays may generally be produced using mechanical synthesis methods or light directed synthesis methods, which incorporate a combination of photolithographic methods and solid phase oligonucleotide synthesis. (See Fodor et al., *Science*, 251:767-777, (1991)). The immobilization of arrays of oligonucleotides on solid supports has been rendered

possible by the development of a technology generally identified as "Very Large Scale Immobilized Polymer Synthesis" (VLSIPS™) in which, typically, probes are immobilized in a high density array on a solid surface of a chip. Examples of VLSIPS™ technologies are provided in US Patents 5,143,854 and 5,412,087 and in PCT Publications WO 90/15070, WO 92/10092 and WO 95/11995, which describe methods for forming oligonucleotide arrays through techniques such as light-directed synthesis techniques. In designing strategies aimed at providing arrays of nucleotides immobilized on solid supports, further presentation strategies were developed to order and display the oligonucleotide arrays on the chips in an attempt to maximize hybridization patterns and sequence information. Examples of such presentation strategies are disclosed in PCT Publications WO 94/12305, WO 94/11530, WO 97/29212 and WO 97/31256.

Preferred embodiments of the present invention include several methods and systems to detect the magnetic signal of a magnetic label that is attached to a biomolecule. Although many biological molecules incorporate iron, biological materials generally exhibit no net magnetic field. (See Stryer, L., *Biochemistry* (1993)). Accordingly, the magnetic signal generated by a magnetic label attached directly or indirectly to a biomolecule is accurately measured and characterized with a high degree of sensitivity and little background noise.

For embodiments that simply detect the presence of a magnetic label on a support (and, hence, whether the biomolecule is attached to a magnetic label), a magnetic sensor such as an inductive read head (e.g., the read head used in a Toshiba model KT-53 stereo cassette) can be used. In contrast, when a relatively precise measurement of the strength of the magnetic field generated by the magnetic label is desired to ascertain not simply the presence of the magnetic label attached to a biomolecule on a support but also the location of the biomolecule, the sensor is desirably a magnetoresistive (MR) read head, as examples only, the read heads used in certain existing disk drives and/or the MR heads made by IBM of Armonk New York or Eastman Kodak Co. of Rochester New York and disclosed by Smith et al. in *J. App. Physics* 69(8):5082 (1991). In some embodiments, the invention uses a MR sensor that is embedded in a chip, wherein the chip has a surface to accommodate the deposit of

biomolecules. In other embodiments, the sensor may be a magnetic force microscope, SQUID sensor, metal film Hall-effect device, or a ultra-high sensitivity susceptometer (for sensing paramagnetic and superparamagnetic markers), such as the device disclosed by Slade et al. in *IEEE Transactions on Magnetics*, 23 (5):3132 (1992).

5           In one embodiment, a sensitive giant magnetoresistive ratio sensor on a solid-state chip ("GMR Sensor") is used in a magnetic detection system to identify the presence of a magnetic label attached to a biomolecule. The GMR sensor, which may run on very low wattage, is a rugged solid state chip which is mass produced inexpensively. The utility of the GMR based sensor is highlighted in one respect by its  
10           sensitivity to small changes in a nearby magnetic field. GMR materials exhibit an order of magnitude greater sensitivity to changes in magnetic field strength than standard anisotropic magnetoresistive materials and saturate at larger fields yielding an improved dynamic range. (See Daughton, J. et al., *IEEE Trans. Mag* 30 (1994), Barnas, J. et al. *PhysiRevi B* 42:8110(1990)).

15           A GMR sensor may be used in the configuration illustrated in Figure 1. A commercially available sensor 1 (model T15, Nonvolatile Electronics Inc., Eden Prairie MN) is coupled to a power supply 2. The sensor 1 advantageously includes biasing magnets for producing an applied biasing magnetic field 3. The input voltage on line 4 and the output of the sensor on line 5 are routed to an operational amplifier 6, and the  
20           output signal 7 is measured. This output signal 7 will vary with variations in the intensity of an externally applied magnetic field 8.

25           A generous dynamic range is obtained by the GMR sensor because the final voltage output depends on the sensitivity and range of the GMR sensor chip, the applied magnetic field, and the input voltage. That is, for any given GMR chip both the offset magnetic bias and the input voltage are easily manipulated to allow for a wide range of detection sensitivity.

30           GMR materials may be composed of alternating 15-40 Å layers of ferromagnetic metals such as CoFe, NiFeCo and alloys such as CuAgAu. To make a sensitive magnetic sensor, the GMR materials may be etched into four resistors on a chip hooked together in a Wheatstone bridge with two of the resistors shielded from magnetic fields. When an external magnetic field is applied, the resistance of the two unshielded GMR

material resistors changes and unbalances the bridge. When an input voltage is put across the bridge, the output voltage increases with the application of a magnetic field. The output voltage is desirably read directly or, for small applied fields, the voltage deviation from the offset voltage is amplified with a commercially available op-amp, as illustrated in Figure 1. The output voltage is preferably displayed using a digital oscilloscope program running on a personal computer, for example. A desirable detection system is disclosed in the published PCT application having International Publication No. WO 96/05326 to Fox, the disclosure of which is incorporated by reference herein in its entirety.

In one embodiment of the invention, the measured magnetic signal is used not only to determine the presence of magnetic label but also to distinguish between different magnetic labels. One of ordinary skill in the art will appreciate generally that different magnetic materials have different magnetic properties. In addition to the fundamental classes of magnetic behavior mentioned above, such as paramagnetism, diamagnetism, ferromagnetism, and the like, different materials within each class have distinguishable magnetic characteristics. For example, ferromagnetic materials exhibit hysteresis in the presence of a varying applied magnetic field. This is illustrated in Figure 2, which shows a graph of magnetization (M) versus applied magnetic field intensity (H) for a hypothetical ferromagnetic material. If an unmagnetized sample of ferromagnetic material is subjected to an increasing applied magnetic field intensity, the magnetization of the material will increase along line 9 of Figure 2. As the field strength is increased, the material reaches a saturation magnetization 10 and no further magnetization takes place as the applied field is increased. Following saturation, if the applied field is slowly reduced, the magnetization of the material will also be reduced. Upon return to zero applied field, however, a remnant magnetization 11 will remain. If the direction of the applied field is then reversed and increased slowly from zero in the opposite direction, the remnant magnetization will be reduced as the material begins to re-orient in the new direction of the applied magnetic field. The applied field strength required to eliminate the remnant magnetization so that the material is demagnetized is known as the coercive force 12. If the field is increased still further in the opposite direction, the material will become increasingly magnetized in that direction, until



saturation is again reached, but in the opposite direction. Reducing the field to zero again results in a remnant magnetization of the same magnitude as the first, but of the opposite polarity. This process of magnetization under an applied field defines a “hysteresis loop” that is characteristic of the material. The three parameters of the hysteresis loop described above, saturation magnetization, remnant magnetization, and coercive force, are each different for different types of ferromagnetic material, and thus, magnetic probes or labels made from different types of material may be distinguished based on these differing magnetic properties.

The present invention also includes methods and systems to assess ratios of these parameters. For example, the ratio of remnant magnetization to the saturation magnetization is known as the “remnant squareness” of the hysteresis loop. The slope of the M-H curve when the hysteresis loop crosses zero magnetization (i.e., at the coercive force designated 12 in Figure 2) is also a characteristic of the material. Another parameter known as “loop squareness” approaches 1 as the hysteresis curve at this point becomes increasingly vertical. These other parameters derived from the hysteresis curve may be especially useful in differentiating magnetic labels, as the measured numerical value of a ratio of measurements or a rate of change of magnetization can be less dependent on the concentration of label in the sample being measured.

In Figure 3, a graph illustrating magnetization as a function of applied field intensity is provided for two different materials. This graph shows the upper left quadrant of the hysteresis curve for neodymium iron boron 13, and for samarium cobalt 14. It can be seen from examination of this Figure that the neodymium iron boron material has a higher saturation magnetization, lower coercive force, and steeper slope at zero magnetization. These features may be used to distinguish the presence of magnetic labels made from different materials.

Embodiments of the present invention measure the magnetization of a selected sample material as a function of applied magnetic field strength. From these measurements, aspects of the hysteresis loop exhibited by the sample are determined. Three types of equipment frequently used to characterize the magnetic properties in materials are the 60-Hz *M-H* looper, the toroidal B-H looper, and the vibrating sample

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magnetometer (VSM). Any of these commercially available instruments, or other comparable equipment or systems, can be used to measure magnetic properties of labels. Therefore, a sample containing a magnetic label of a first kind is distinguished from a sample containing a magnetic label of a second kind. Typically, and as illustrated by Figure 3, the different labels will have different chemical composition. For example, they can comprise two different iron alloys, or an iron based label and a rare earth element based label. Samples containing these labels will exhibit hysteresis loops having different shapes, and are thus distinguishable with magnetization analysis under an applied external magnetic field. Mixtures of two different labels are also detectable because the sample will exhibit a hysteresis loop having characteristics that are intermediate between the loops exhibited by the two labels individually.

The present invention also includes methods and systems to detect and characterize magnetic labels attached to a biomolecule disposed on a support. Because magnetic labels attached to a biomolecule generate a quantifiable magnetic field, the presence and location of biomolecule on the support can be determined. For example, in one preferred embodiment, when a support having a biomolecule attached to a magnetic label is juxtaposed with a magnetic sensor and moved past the magnetic sensor, the magnetic field of the attached magnetic markers variably permeate the sensor and thereby cause the sensor to generate a detection signal. For label characterization, an external magnetic field is applied, and sample magnetization is measured at a plurality of applied field strengths. When detecting the presence of label, the support is preferably closely juxtaposed with the sensor and, more preferably, the substrate is distanced from the sensor, by way of examples only, by only a few microns or less, so as to improve the sensitivity of detection. In other embodiments, the support and the sensor may be integrated. The sensor is electrically connected to a signal processor that receives the detection signal and generates a signal representative of the amount of magnetic label present.

The signal processor includes signal processing circuitry known in the art for processing signals from magnetic sensors, as well as a correlator for generating a biomolecule concentration based upon the detection signal from the magnetic sensor. For example, the correlator can be a programmable chip or a microprocessor having

software which interprets the magnetic signal information to calculate and display biomolecule concentration. Desirably, the correlator is calibrated to generate accurate biomolecule concentration by means well-known in the art, e.g., by passing several supports having known quantities of a biomolecule deposited thereon next to the sensor and adjusting the resulting detection signals to the known concentrations. Additionally, the signal from the signal processor can be sent to an output device.

If desired, the present invention may also include a transporter and a support that can be positioned on the transporter to move the support past the sensor. In one embodiment, the sensor is moved past the substrate in a raster-scan type motion to generate a two-dimensional data output, e.g., an image, having an "x" dimension and a "y" dimension. Further, the two-dimensional data output can be transformed into a three-dimensional output wherein the third dimension ("z" dimension) represents magnetic signal intensity.

Preferred embodiments of the invention provide methods and systems to attach a biomolecule with a detectable magnetic label. In some embodiments, a biomolecule is directly attached to a magnetic label (e.g., by an interaction with a ferrofluid) and in others the biomolecule is indirectly attached to a magnetic label (e.g., by an interaction with a magnetically-labeled protein, as described below). Positively charged ferrofluids offer many advantages over other types of magnetic labels. These ferrofluid magnetic labels have no special storage, handling or disposal requirements and are relatively easy to fabricate. Ferrofluids are commercially available and ferrofluids having many different types of magnetic particles and, thus, different magnetic properties, can be custom-made and obtained through Ferrofluidics, Nashua, NH. Each particle in a ferrofluid has an intrinsic magnetic moment that can be aligned and accentuated with the application of an external orienting field. In solution, ferrofluids exist in a colloidal state but when ferrofluids bind to a biomolecule their colloidal properties diminish. Additionally, ferrofluids in a colloidal state are not strongly attracted by a magnet, however, when bound to a biomolecule, the magnetic properties of a ferrofluid permit magnetic attraction. A ferrofluid's loss of colloidal properties upon binding to a biomolecule and the ability to attract biomolecules bound to a ferrofluid with a magnet

are exploited by the invention to separate ferrofluid-bound biomolecules from biomolecules and ferrofluid which have not interacted.

In some embodiments, a ferrofluid is attached to a biomolecule (e.g., a probe for the detection of a specific nucleic acid sequence or protein domain) and separated from unbound biomolecules and unbound ferrofluids in many ways. In one embodiment, a biomolecule is contacted with a ferrofluid for a time sufficient to allow the magnetic label to interact with the biomolecule. Subsequently, centrifugation is performed to loosely pellet the biomolecules having attached magnetic labels. The supernatant is removed and the pellet is resuspended in distilled water or a suitable buffer. This "washing" procedure is desirably performed several times so as to effectively remove all the unbound ferrofluid and unbound biomolecules. The unbound colloidal ferrofluid and unbound biomolecules remain in solution, while the ferrofluid bound to the biomolecule is pelleted and, thus, separated from the unbound ferrofluid and unbound biomolecules.

In another embodiment, a magnet is used to separate biomolecules bound to a ferrofluid from unbound ferrofluids and unbound biomolecules. As above, a biomolecule is contacted with a ferrofluid for a time sufficient to allow the magnetic label to interact with the biomolecule. Subsequently, a magnet is applied, for example, to the side of the vessel housing the biomolecule and ferrofluid, and the biomolecules having attached magnetic labels are aggregated near the magnet. The supernatant is carefully removed and the magnetic aggregate is resuspended in distilled water or a suitable buffer. This "washing" procedure is desirably performed several times so as to effectively remove all the unbound ferrofluid and unbound biomolecules. The unbound colloidal ferrofluid and unbound biomolecules remain in solution, while the ferrofluid bound to the biomolecule is aggregated and, thus, separated from the unbound ferrofluid and unbound biomolecules.

As indicated above, embodiments of the present invention include the use of positively charged ferrofluid colloids to directly label a probe biomolecule. Such techniques can also be used in the invention to label a biomolecule disposed on a support so as to detect its presence and location. The detection of a biomolecule disposed on a support, for example, is accomplished by applying the ferrofluid to the

biomolecule, washing away unbound or non-specifically bound ferrofluid, and detecting the magnetic signal generated by the bound magnetic label. From the information generated by the magnetic signal from the support, the presence and location of the biomolecule are determined.

5           The invention also includes methods and systems to attach a magnetic label to a target biomolecule indirectly by binding a magnetically labeled secondary molecule to the target biomolecule. In addition to the use of magnetically labeled probe biomolecules to detect specific sequences or proteins, as will be discussed below, magnetically labeled secondary molecules, as examples only, nucleic acids or proteins,  
10           are used to detect biomolecules disposed on a support. As examples, and without limitation, in some embodiments a magnetic label is attached to a nucleic acid which interacts with a protein binding domain such as found in transcription factors or other nucleic acid binding proteins. In other embodiments, a magnetic label is attached to a protein which interacts with a modified nucleotide within a nucleic acid sequence or a  
15           modified domain of a protein. In the latter instance, magnetically labeled antibodies specific for modified biomolecules, such as dinitrophenol (DNP), isopentenyl -6-adenosine (I<sub>6</sub>A), and biotin, are used. Additionally, biotin residues on a nucleic acid or protein are readily detectable with embodiments that use magnetically labeled avidin, streptavidin, monomeric avidin, and derivatives or modifications of these proteins.  
20           Accordingly, these proteins are preferably labeled with a ferrofluid and are separated from unbound protein and ferrofluid by the methods detailed above, however, several commercially available magnetic antibodies and magnetic avidin and streptavidin are available.

25           Embodiments of the invention also include methods and systems to detect specific biomolecules within a population of heterogeneous biomolecules disposed on a support. One of ordinary skill in the art will appreciate that many conventional approaches to specific nucleic acid detection, such as Northern and Southern hybridization, and specific protein detection, such as Western blotting and immunoprecipitation, are adaptable for use with embodiments of the present invention.  
30           In some embodiments of the present invention, a non-magnetic colloid or other blocking agent which binds to single stranded nucleic acid or non-specific binding sites on a

target biomolecule are added. Non-magnetic colloids, such as silver stain, and blocking agents, such as Salmon sperm DNA, carrier RNA, bovine serum albumin, ovalbumin, and casein, are added to reduce non-specific binding of probes and background noise.

One embodiment of the invention identifies a specific biomolecule (e.g., proteins or nucleic acids) within a population of heterogeneous biomolecules is as follows: First, a sample having a target biomolecule, among a heterogeneous population of biomolecules, is disposed on a support. The target biomolecule on the support is then contacted with a magnetically-labeled probe biomolecule that interacts with the target biomolecule. The unbound and nonspecifically bound magnetic probe is removed by washing in a suitable buffer, and the bound magnetic signal is measured and characterized with a magnetic sensor, as described above. Accordingly, the presence of a magnetic signal at a specific location on the support identifies the presence of the target biomolecule. Alternatively, as discussed above, one or several different probe biomolecules can be disposed on a support at different locations so as to create an addressable array that is used to detect the presence of one or more target biomolecules in a preparation of biological sample. Magnetically labeled biomolecules present in the biological sample are applied to the array, the support is washed so as to remove unbound and nonspecifically bound biomolecules, and the magnetic signal that remains on the support is detected using a magnetic sensor, and the presence of the target biomolecule in the biological sample is identified.

In other embodiments, many different probes or biological samples or both are screened at the same time. By using a method referred to as "multiplexing", the invention screens biomolecules present in several biological samples, including samples from different individuals, against a battery of probe biomolecules in the same reaction to determine predispositions to disease, genetic typing, and forensic identification, as examples.

In one embodiment, an addressable array is constructed wherein many different probe biomolecules (e.g., nucleic acid probes or antibodies or other types of protein probes) are disposed on a support at locations that are separate from one another and readily identifiable. The locations and identities of the probe biomolecules on the support are recorded (e.g., on a recordable computer media such a computer disk, hard

drive, CD ROM, DVD ROM, or other recordable media as known in the art). Biological samples from three individuals, for example, having biomolecules that correspond or are detectable by probes on the array if the target biomolecule is present are obtained and prepared, according to conventional techniques in hybridization or blotting or both. The three different biological samples are separately labeled with different magnetic labels (e.g., ferrofluids) such that the first is labeled. The magnetically labeled biological samples are washed so that only specifically bound magnetically labeled biomolecules remain in the samples and the samples are pooled.

The pooled sample now comprises the biomolecules of three different individuals and three different magnetic labels. The pooled sample is then contacted to the array under conditions which allows for specific binding of the probe biomolecules to any target biomolecules that may be present in the three different samples. The unbound and nonspecifically bound biomolecules are removed by washing in a suitable buffer, and the array is passed before a magnetic sensor which characterizes and measures the magnetic signals bound to the support in an applied magnetic field, for example. Because each of the three different magnetic labels has a magnetic particle that has a unique magnetic signal (e.g., hysteresis curve shape and slope, saturation magnetization, remnant magnetization, coercive force, etc.), the identity of the presence or absence of each type of magnetic particle can be accomplished in the same reaction. Thus, the detection of one, two, or three magnetic signals from one or more locations on the array can be accomplished using this embodiment of the present invention, and the ability to rapidly screen several individuals for many different indicators for disease and genetic composition has been accomplished.

As one of ordinary skill in the art will readily recognize embodiments of the multiplexing method of the invention can be used to screen a single individual against a battery of magnetically labeled probes. Accordingly, different types of probe biomolecules (e.g., protein or nucleic acid) are labeled with unique magnetic particles so that the presence of multiple probe biomolecules bound to target biomolecules (e.g., protein or nucleic acid) from a biological sample from the individual can be accomplished. A biological sample from the individual to be screened is obtained and prepared and the biomolecules to be probed are disposed on a support. The various

magnetically labeled probes are contacted with the biomolecules on the support, the unbound and nonspecifically bound probes are removed by washing, and the magnetic signals are detected and resolved, as described above.

5 Additionally, multiplexing embodiments of the invention may perform dynamic competitive binding studies. In one embodiment, a target biomolecule (e.g., protein or nucleic acid) is disposed on a support or several target biomolecules (e.g., proteins or nucleic acids) are disposed on a support so as to create an addressable array. Two or more probe biomolecules (e.g., protein or nucleic acid) that are thought to compete with one another for the same target biomolecule are joined with different magnetic labels, as  
10 described above. Thus, the competing probe biomolecules have magnetic labels that provide magnetic signals which can be resolved from one another. The competing probe biomolecules are contacted with the target biomolecule(s) under conditions that allow for binding and the unbound or nonspecifically bound probes are removed by washing. The support is then passed before a magnetic sensor, the magnetic signals are  
15 detected and resolved, and competitive inhibition is determined. In additional embodiments, the support and sensor may be integrated. Desirably, the binding kinetics of the competition are performed over time at varying concentrations of the different probes. By using multiplexing, the competitive inhibition of many different probe biomolecules can be determined in the same reaction.

20 The invention includes embodiments which identify an interaction of a probe with a target biomolecule in a heterogeneous sample solution. In additional embodiments, the invention detects the presence of target biomolecules in solutions, such as biological fluids obtained from a test subject. An embodiment to rapidly detect one or more target biomolecules in a solution uses colored beads onto which one or  
25 more probe biomolecules are joined, referred to as a "colored bead-probe reagent". For example, a colored bead-probe reagent is generated by an approach described above, and magnetically labeled biomolecules that are obtained and prepared from a biological sample from a subject are contacted with the colored beads that are joined to the probe biomolecules. If a target biomolecule is present in the biological sample, then a colored  
30 bead-probe-target biomolecule complex will form. Since the target biomolecule is labeled with a magnetic probe, the colored bead-probe-target biomolecule complex can



be separated from the unbound colored beads joined to probe biomolecules with a magnet. In a preferred embodiment, a bar magnet is applied to the side of the reaction vessel to aggregate the colored bead-probe-target biomolecule complexes and quickly identify the presence of the target biomolecule in the biological sample. By using several different colored beads, each color having a probe biomolecule that detects the presence of a different target biomolecule, multiple target biomolecules can be detected in the same reaction. A colored mixture of beads obtained in a given reaction can be compared to a color chart to rapidly identify the presence and amount of target biomolecule in a biological sample.

Additionally, in some embodiments, an optical device, such as spectrophotometer or a photodiode CCD camera, is employed to more accurately measure the binding of a probe biomolecule to a target biomolecule and to determine the relative amount of target biomolecules in the reaction. For example, after the aggregated colored bead-probe-target biomolecule complexes are separated from colored bead-probe reagents that failed to bind to a target biomolecule, the colored bead-probe-target biomolecule complexes are resuspended in a solution and the absorbance of light is determined by conventional spectroscopy. By using colored bead standards (e.g., a known quantity or mixture of colored beads having reproducible absorbance values), one of ordinary skill in the art can accurately determine the amount of colored bead-probe-target biomolecule complex present in the solution and, thereby, determine the amount of target biomolecule present in the biological sample. Similarly, varying shades of colored beads are detectable when more than one color of bead-probe reagent is used to detect multiple target biomolecules and the presence of the various target biomolecules is determined by comparing absorbance readings at different wavelengths and comparing these values to standards.

Additional embodiments may include, as examples only, fluorescent beads or fluorescent cells which produce fluorescent signal ( for example, like green fluorescent protein), with subsequent measurement of fluorescence.

Additional embodiments of the present invention include competitive inhibition assays using multiple colored beads. Accordingly, a magnetically labeled target biomolecule is placed in a solution comprising two or more competing probes (e.g.,

probes that compete for the same binding site) that are joined to different colored beads. Binding is allowed to occur and a magnetic field is applied to the reaction so as to allow for the aggregation and separation of colored bead-probe-target complexes from the colored bead-probe reagent that failed to form a complex with the target biomolecule.

5 The relative binding of each probe is resolved by visually determining the amount of binding of each colored bead, according to a visual inspection of a color chart, or more precise measurements may be obtained by employing an optical device, such as a spectrophotometer or CCD camera, as described above.

10 Another embodiment of the present invention detects target biomolecules in a solution by monitoring magnetic swing time. "Magnetic swing time" means the time it takes for a magnetic compound to reorient itself to a changed magnetic field. For example, when a single magnetic particle is attached to a biomolecule, this magnetic-biomolecule complex will orient itself in a solution according to the magnetic field placed near the complex. If the magnetic field direction is then moved by 90°, a period  
15 of time is required for the magnetic biomolecule complex to reorient itself to the new field position. The measurement of the time it takes for the magnetically-labeled molecule to reorient itself to the changed magnetic field is referred to as magnetic swing time.

20 The present invention measures magnetic swing time to determine when a magnetically labeled biomolecule has complexed with another biomolecule. That is, the binding of a target biomolecule by a magnetically labeled probe molecule provides a greater mass and, thus, a greater swing time than the unbound magnetically labeled probe. By measuring the time that is required for the magnetic particles to reorient themselves to the changed magnetic field, the mass of the magnetically labeled complex  
25 can be determined. For example, if the mass has increased (by binding the target biomolecule), magnetic swing time will also increase when all of the other factors are held constant.

30 In one embodiment, a target biomolecule (e.g., a protein or nucleic acid) is suspended in a suitable buffer. In a separate vessel, a magnetically labeled probe molecule which interacts with the target biomolecule is suspended in a suitable buffer. A strong magnetic field is applied to the magnetically labeled probe molecule so as to

orient the magnetic label to the magnetic field. Once the initial magnetic field orientation is established, the position of the magnetic field is shifted (e.g., by 90°) and the time that is required for the magnetically labeled probe to reorient itself to the magnetic field is measured. Because the strength of the magnetic field is constant and the mass of the magnetically labeled biomolecule probe is known, the acceleration of the magnetically labeled biomolecule, as a function of time, is determined.

Next, the magnetically labeled biomolecule is brought in contact with the target biomolecule so as to permit the formation of a target biomolecule-probe complex. After sufficient time for binding is allowed and all unbound probe is removed from the vessel by washing, a strong magnetic field is applied so as to orient the target biomolecule-probe complex. Once orientation to the magnetic field is achieved, the magnetic field is shifted (e.g., by 90°) and the time to reorient to the new field location is measured and recorded. A difference between the magnetic swing of the probe biomolecule in an applied magnetic field and the target biomolecule-probe complex in an applied magnetic field indicates that a binding of the target biomolecule and the probe biomolecule has occurred.

Although embodiments described above use a magnetically labeled biomolecule probe, other embodiments may use a bead that polarizes light. In this embodiment, calcite beads can be used, but fluorescent beads or fluorescent cells which emit polarized light could be employed. Next, the magnetically labeled biomolecule is brought in contact with the target polarized beads biomolecule so as to permit the formation of a target biomolecule-probe complex. After sufficient time for binding is allowed and all unbound probe is removed from the vessel by washing, a strong magnetic field is applied so as to orient the target biomolecule-probe complex. Polarized light orientation and intensity are measured. The magnetic field is shifted (e.g., by 90°) and change in the polarized light orientation and intensity are measured and recorded. A difference between the orientation and intensity of the light indicates that a binding of the target biomolecule and the probe biomolecule has occurred. The embodiments described above provide another very sensitive method for determining that a binding interaction between two biomolecules has occurred.

5 The invention includes methods and systems to enhance the binding of a probe biomolecule to a target biomolecule and to reduce non-specific binding and background noise. In one embodiment, a target biomolecule (e.g., a nucleic acid or protein) is disposed on a support and is contacted with a probe biomolecule having an attached magnetic label, as described above. To enhance binding, a magnetic field is applied to regions of the support near the target biomolecule so as to induce the magnetically labeled probe biomolecule to move toward and concentrate at the position corresponding to the target biomolecule. In this manner, a greater binding to the probe biomolecule is obtained. Additionally, an electric field is applied in conjunction with the magnetic field so as to enhance the movement toward and concentration at the site near the disposed target biomolecule. In another embodiment, an electrical field or a magnetic field or both are applied to the support after binding of the target biomolecule by the magnetically-labeled probe so as remove or separate from the target biomolecule any unbound or non-specifically bound probe biomolecule.

10 In some embodiments, a pulsing electrical or magnetic field is used to move the probe biomolecule toward the target biomolecule and concentrate it at that site or, alternatively, to induce the unbound probe biomolecule or non-specifically bound probe biomolecule to move away from the target biomolecule. By applying the approaches described above, a magnetically labeled probe can be concentrated at a site near the target biomolecule and thereby increase the kinetics of binding, and unbound and non-specifically bound probe can be separated from the specifically bound probe so as to reduce background.

15 Preferred embodiments of the invention may separate magnetically labeled biomolecules on the basis of mass by applying a magnetic field. In one embodiment, the invention provides methods and systems that separate magnetically labeled biomolecules according to their mass in an applied magnetic field. Biomolecules are first labeled with a magnetic marker, preferably a ferrofluid, for example by the approaches detailed above. Once the biomolecules are magnetically labeled, they are suspended in a solution (e.g., a suitable buffer) and a magnetic field is applied to the sample. Because the amount of ferrofluid which binds to the biomolecule is directly proportional to the mass of the biomolecule, molecules with greater mass have a greater

magnetic potential than smaller molecules. Accordingly, magnetically labeled biomolecules are separated according to their mass by applying a strong magnetic field.

The following examples are provided for exemplary purposes and are not intended to limit embodiments of the present invention.

5

## EXAMPLE 1

In this example, the present invention detects the presence and location of a nucleic acid disposed on a support by means of a magnetic label attached to a nucleic acid. As an example, one microliter containing 100ng plasmid DNA (pHIS,  $\approx$  3700 base pairs/molecule) are spotted on a positively charged nylon membrane (Hybond, Amersham) that is subsequently cut into 1cm X 4cm strips. The strips are air dried for 10 minutes and each strip is dipped in distilled water for 10 seconds. Next, the strips are contacted with a ferrofluid (EMG607; Ferrofluidics, Nashua NH) for two minutes, then washed for 2 minutes in distilled water and air dried.

Samples are measured using a GMR sensor (T15 model; Nonvolatile Electronics Inc., Eden Prairie MN) with bias-magnets, and the voltages are recorded by a PC after analog processing and 12 bit A/D conversion. Output from the prototype magnetic detection system unit for the detection of nucleic acids is shown in Figure 4. The magnetism of the DNA/ferrofluid filters is also measured with a vibrating sample magnetometer (VSM, Digital Measurement Systems Inc.) to verify and calibrate the results. The VSM determines the actual emu generated at the surface of the sample, whereas the GMR sensor determines the relative emu. Triplicate samples, prepared identically to the one used to generate the GMR sensor data shown in Figure 4, yield an average of  $4.5 \times 10^3 (\pm 0.7)$  emu. From this data it may be determined that one relative magnetic unit (RMU) equals  $\approx 10^5$  emu.

As another example, similar concentrations of double stranded plasmid DNA (dsDNA) or single stranded DNA are spotted on a membrane and the GMR sensor reader displays a linearity of response on a semi-log plot, as shown in Figure 5. A sensitivity of detection on the order of 2 ng/ul for both ssDNA and dsDNA is obtained. In a third example, the detection of total RNA at a similar sensitivity is demonstrated, as shown in Figure 6. The data presented above show that a GMR sensor can detect over

three orders of magnitude (microgram-to-nanogram) of dsDNA, ssDNA, and total RNA in a very small volume of sample.

#### EXAMPLE 2

5 In this example, the invention detects a protein disposed on a support. In this example, the invention detects the presence and location of a protein disposed on a support by means of a magnetic label attached to a protein. In one example, 1ul of a solution containing either mouse IgG or goat IgG is spotted on a membrane that is subsequently cut into 1cm X 4cm strips. The strips are then air dried and each strip is dipped in distilled water. Next, the strips are contacted with 2 ul of ferrofluid (EMG607; Ferrofluidics, Nashua NH). The samples are then washed, allowed to air dry, and are measured using a GMR sensor (T15 model; Nonvolatile Electronics Inc., Eden Prairie MN) with bias-magnets, and the voltages were recorded by a PC after processing as in Example 1. The results show that ferrofluid efficiently labeled mouse IgG and goat IgG which was disposed on a support and the proteins are effectively detected using the GMR sensor.

#### EXAMPLE 3

20 The invention also comprises hybridization using magnetically labeled nucleic acid probes are provided. In this example, the invention uses a magnetic label attached to a nucleic acid probe to detect a target nucleic acid disposed on a support. A first oligonucleotide of 52 nucleotides (T54) is used as a target nucleic acid and a second complementary oligonucleotide (T55), bound with a ferrofluid, was used as a probe nucleic acid. One microliter of the target nucleic acid T54 (at 0.5 ug/ul) is spotted on a nylon membrane and is crosslinked with UV light (autolink setting; Strat linker 2500; Stratagene). Subsequently, the membrane is washed briefly with water and air dried.

25 The probe nucleic acid is made by incubating 2ug of a complementary oligonucleotide, T55, with ferrofluid (1:1 (v:v) in 10 :l total volume). Unbound T55 is separated and removed from the ferrofluid conjugated T55 by washing with water, as described above.

30 The hybridization is conducted as follows. The ferrofluid-labeled probe nucleic acid is suspended in 3ml of a 1X SSC solution (Sambrook, J. et al., *Molecular Cloning, A Laboratory Manual* (1989)) and is placed in a 15ml conical tube containing the support having the target nucleic acid. The hybridization is conducted in an oven at

50°C for 16 hours. A negative control is run in parallel using T55 as both the probe and target nucleic acid. The filters are removed, washed in 1X SSC, and air dried. The relative magnetic units are then determined using the GMR sensor, as described in Example 1. The sample having the T54 target nucleic acid and the T55 probe nucleic acid yield an average of 710 ( $\pm 64$ ) RMU, whereas, the sample having the T55 target nucleic acid and the T55 probe nucleic acid had an average of 360 ( $\pm 80$ ) RMU. Moreover, when the salt concentration in the hybridization buffer is lowered, a decrease in signal for the sample having the T54 target nucleic acid bound with T55 probe was observed, indicating strand displacement. In contrast, a decrease in signal is not observed for the control when the salt concentration was lowered. These results demonstrate the invention's ability to perform nucleic acid hybridization using a ferrofluid-bound nucleic acid probe.

#### EXAMPLE 4

The invention also detects a target nucleic acid by using a biotinylated nucleic acid probe and a ferrofluid-labeled streptavidin marker. In this example, the invention uses streptavidin conjugated with a magnetic label to identify the presence and location of a biotinylated nucleic acid probe hybridized to a target nucleic acid disposed on a support. The probes for these experiments comprise a DNA-biotin-streptavidin-magnetic bead complex. Biotinylated 40-mer oligonucleotides complementary to a regions of the 8 phage genome are used. There are many custom service companies for oligonucleotide synthesis but, desirably, the nucleic acid probes are made on the premise using a Milligen Cyclone Plus synthesizer at the .012 micromole scale. Commercially available modification chemicals are used to quantitatively biotinylate the oligonucleotide directly on the synthesis column (Cruachem). There are also several commercial sources of streptavidin magnetic beads (MPG, Dynal, Promega or Boehringer Mannheim). In order to reproduce an optimum coupling efficiency, various dilutions of the 5um beads will be contacted with the biotinylated oligonucleotide. Desirably, the highest magnetic concentration is sought so as to minimize the possibility that any given bead will have more than one oligo attached. Custom preparations of

magnetic beads, having a single streptavidin molecule per bead, are also obtainable. (Bangs Labs, Fishers, IN).

DNA from 8 phage is isolated and a region encoding the D gene is used as the target nucleic acid. (See Mikawa et al., *J. Mol. Biol.* 262:21 (1996) for a description of suitable target nucleic acid sequences and complementary probe nucleic acid sequences). There are a number of well-known chemicals used to isolate viral RNA or DNA. (Sambrook, J. et al., *Molecular Cloning, A Laboratory Manual* 1989)). Phenol, for example, denatures the coat proteins of virus and liberates the nucleic acids inside. The proteins aggregate at the phenol/water interface and the nucleic acid remains in the aqueous phase. Phenol is, however, a moderately caustic chemical and several methods that rely on less harsh agents have been developed. Numerous formulations based on combinations of detergents (SDS, SLS, Nonidet P40, reductants (DTT and beta-ME), proteases (Proteinase K, pronase) and chaotropics (guanidine, guanidinium thiocyanate) have also been published. (Sambrook, J. et al., *Molecular Cloning, A Laboratory Manual* (1989), Luria, S.E. et al., *General Virology* and Calendar, R., *The Bacteriophages* (1988)).

Once the phage DNA is isolated, it is spotted at various concentrations on a nylon membrane and crosslinked with a standardized dose of UV light (1200 units in a Stratalinker). The filter is also, preferably, prehybridized with a non-magnetic colloid and/or a blocking agent. The filter is then brought to 6X SSC and the magnetically-labeled probe (biotinylated oligonucleotide bound to magnetically-labeled streptavidin) is added. Hybridization is conducted for 16 hours at 20°C below the calculated  $T_m$ . After washing, the sample is measured for magnetic activity with a GMR sensor, as described above. As a negative control, 8 DNA is spotted on the filter and hybridized with a non-complementary oligonucleotide coupled to a magnetic bead. As a positive control, a biotinylated oligonucleotide probe is labeled with a streptavidin-alkaline phosphatase conjugate and the filter is developed with standard precipitating substrates. These results demonstrate that nucleic acid hybridization using magnetically-labeled streptavidin molecules bound to biotinylated nucleic acid probes can be accomplished.



## EXAMPLE 5

The invention also uses nucleic acid hybridization that exploits the magnetic signal generated by a ferrofluid. In this example, the invention uses the increase in magnetic signal obtained by a nucleic acid hybrid over a single stranded nucleic acid to identify the presence and location of the nucleic acid hybrid. In this example, a first oligonucleotide of 52 nucleotides (T54) is used as a target nucleic acid and a second complementary oligonucleotide (T55), is used as a probe nucleic acid. One microliter of the target nucleic acid T54 (at 0.5 ug/ml) is spotted on a nylon membrane and is crosslinked to the membrane with UV light (autolink setting; Stratalinker 2500; Stratagene). Subsequently, the membrane is washed briefly with distilled water and is allowed to air dry. The unlabeled probe nucleic acid (T55) is suspended in 3ml of a 1x SSC solution (Sambrook, J. et al., *Molecular Cloning, a Laboratory Manual*, (1989)).

The support having the target nucleic acid (T54) and the 3 ml of 1x SSC solution containing the unlabeled probe nucleic acid (T55) are combined in a 15ml conical tube. The hybridization is conducted in an oven at 50°C for 16 hrs. The negative control for the experiment is run in parallel, and uses T55 as both the probe and target nucleic acid. The experimental and control filters are removed from the oven, washed in 1x SSC, and air dried. Next, the supports are placed in a 15ml conical tube containing a 3ml suspension of ferrofluid (1:1 v/v). Binding of the ferrofluid to the nucleic acids present on the supports is conducted for 5 minutes. The supports are then removed and washed in 1x SSC and air dried. The magnetic signal present on the supports is then determined using a GMR sensor, as described above.

The sample having the T54 target nucleic acid and the T55 probe will have a greater average RMU than the sample having the T55 target nucleic acid and the T55 probe. Furthermore, another washing of the membrane with a lower salt concentration (e.g., 0.1 SSC) will promote strand displacement and a decrease in signal for the support having the T54 target nucleic acid will be observed. These results demonstrate that a conventional nucleic acid hybridization with an unlabeled probe can be performed and sensitive detection of nucleic acid hybrids, by using a ferrofluid after hybridization, can be accomplished.

## EXAMPLE 6

The present invention also detects a target nucleic acid by using a nucleic acid probe attached to a colored bead and a ferrofluid-labeled target nucleic acid. In this example, the invention rapidly identifies the presence of a specific nucleic acid sequence in a solution. A biotinylated oligonucleotide probe complementary to a region of the 8 phage genome is used. A biotinylated 40-mer probe which complements a region of 8 can be made as described in Example 4. A colored bead onto which streptavidin is attached is used to sequester the biotinylated oligonucleotide. Streptavidin beads are common in the art and the ability to generate a colored variety is straightforward. Many activatable linkers, some of which were described above, are available and can be used to attach streptavidin to a colored bead. Further, custom preparations of streptavidin magnetic beads can be obtained through several suppliers including Bangs Labs, Fishers, IN .

The streptavidin beads are coupled to biotinylated oligonucleotides in TBS (Sigma T6664) and are, desirably, blocked with a non-magnetic colloid or a blocking agent or both, as described previously, overnight on a shaker. The beads are collected, washed in TBS, and the unbound biotinylated oligonucleotide is removed. The amount of biotinylated oligonucleotide which binds to the streptavidin beads can be determined by using radiolabeled oligonucleotides of a known specific activity.

Once the colored beads have been coupled to the biotinylated oligonucleotide, this immobilized probe is suspended in 3ml of a hybridization solution having DNA isolated from the 8 phage that has been magnetically labeled. Many hybridization solutions are known (see e.g., Sambrook, J. et al., *Molecular Cloning, A Laboratory Manual* (1989)) and a 1X SSC solution with a blocking agent such as Salmon Sperm DNA or carrier RNA is desirable. Prior to hybridization, the isolated DNA is attached to a magnetic label (e.g., a ferrofluid) by using the approaches detailed above. Hybridization to the immobilized probe which complements a region of the D gene of the 8 phage, is conducted at 37°C for 16 hrs in a 15ml conical tube. Subsequently, the colored beads are brought to the bottom of the conical tube, washed several times in 1x SSC, and are resuspended in a 1x SSC solution. Next, a thin bar magnet is applied to

the side of the conical tube and the colored beads having the biotinylated probe hybridized to a ferrofluid-labeled target nucleic acid aggregate near the magnet. In this manner, the rapid identification of the presence of a target nucleic acid in a solution is determined.

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## EXAMPLE 7

The invention also detects several target nucleic acid sequences in a sample by using multiple magnetically labeled probes. In this example, the invention identifies multiple target nucleic acid sequences in a biological sample having polynucleotides in the same reaction by using multiple magnetically labeled nucleic acid probes. The nucleic acid present in 8 phage are isolated by conventional techniques and are disposed on a support (e.g., membrane or chip). The nucleic acid on the support is then contacted with at least two nucleic acid probes which complement a different nucleic acid sequence present in the phage genome. For example, a first nucleic acid probe complements one region of the gene encoding the D protein and the second nucleic acid probe complements a second region of the gene encoding the D protein.

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The first nucleic acid probe also has a magnetic label that is different, (therefore having a different magnetic characteristic), from the second nucleic acid probe. For example, the magnetic particles for the two probes is made of different iron or transition metal alloys having different hysteresis characteristics. It will be appreciated that more than two nucleic acid probes can be used, in accordance with this embodiment, to detect multiple target nucleic acid sequences present in a biological sample, so long as each nucleic acid probe has a different magnetic label.

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The magnetic labels (e.g., ferrofluids) are attached to the probe nucleic acids according to the approaches detailed above. The support having the 8 DNA is preferably prehybridized in a solution having a non-magnetic colloid and/or a blocking agent for 2 hours at 37°C. The magnetically labeled probes are added to the prehybridized support in a hybridization solution and hybridization is allowed to occur for 16 hours at 37°C. Prehybridization and hybridization solutions are described in Sambrook, J. et al., *Molecular Cloning, a Laboratory Manual* (1989) and the conditions for hybridization may vary depending on the nucleic acid probes used and the nucleotide composition of the target nucleic acid sequences.

After hybridization, the unbound and non-specifically bound probes are removed by washing in a suitable wash buffer (e.g., 2X SSC) and the presence of a hybrid containing the first magnetically-labeled nucleic acid probe and a second hybrid having the second magnetically-labeled nucleic acid probe is detected. If the biological sample contains a nucleic acid sequence which complements the first and/or the second nucleic acid probe, then a magnetic signal which corresponds to the first and/or the second magnetic label will be detected by analyzing, for example, the magnetic hysteresis characteristics of the biological sample. Thus, rapid diagnostic screening using multiple nucleic acid probes having different magnetic labels can be accomplished.

## EXAMPLE 8

The invention also detects a target protein disposed on a support by using a magnetically labeled probe. In this example, the invention specifically identifies proteins disposed on a support by a magnetically labeled probe. In the example, the probe is composed of a mouse anti-Epidermal Growth Factor (EGF) antibody (Sigma E2635) which is attached to a magnetic label (e.g., a ferrofluid). To prepare the probe, the anti-EGF antibody is coupled to a ferrofluid, as described above. The target protein is mouse Epidermal Growth Factor (EGF) isolated from submaxillary glands of mice. (Sigma E6135). Epidermal growth factor (EGF) is a 6kD polypeptide originally discovered and purified from mouse submaxillary glands. (Levi-Montalcini and Cohen, *Ann. N. Y. Acad. Sci.*, 85:324 (1960) and Cohen, *J. Biol. Chem.*, 237:1555 (1962)).

Varied concentrations of the target protein are spotted onto a nylon membrane and the protein is immobilized to the membrane by heating under vacuum. The supports having the bound target protein are placed in a 15ml conical tube and the support is blocked in a solution of 5% w/v BSA in TBS (Sigma) for 2 hrs. at room temperature. Other blocking agents or a non-magnetic colloids or both may be added. The blocked support is then overlaid with 3ml of ferrofluid-labeled anti-EGF antibody suspended in the blocking buffer at an appropriate dilution (generally 1:50 - 1:500). Binding is then allowed to occur for 1 - 3 hrs. at room temperature on a shaker. The support is subsequently washed in TBS and the target protein-probe complex is detected by passing the membrane before a GMR sensor, as described above. Once the magnetic signal is determined, the probe can be stripped using conventional techniques, and the

blot is reprobed with a control antibody (e.g., ferrofluid-labeled anti-bovine IgG) (Sigma B6901), according to the technique outlined above. Alternatively, a side-by-side immunoblot with the control antibody can be performed. After binding of the control antibody is allowed to take place, the support is washed, and the presence of bound  
5 antibody is ascertained by passing the support before a GMR sensor. The results of this example demonstrate that protein identification using a ferrofluid-labeled antibody can be accomplished.

#### EXAMPLE 9

The invention also comprises methods and systems that use immunoblotting  
10 techniques. In this example, the invention provide method and systems that use ferrofluids bound to an antibody-target protein complex to identify the presence of a specific protein in a population of heterogeneous proteins. The probes in this example are the ferrofluid-labeled anti-EGF antibody (experimental) and the ferrofluid-labeled anti-bovine IgG antibody (control), as described above. Total protein is isolated from  
15 mouse submaxillary glands by conventional techniques. Levi-Montalcini and Cohen, *Ann. N. Y. Acad. Sci.*, 85:324 (1960) and Cohen, *J. Biol. Chem.*, 237:1555 (1962). The isolated total protein (5-20 ug per well) is then separated on a 15% SDS/PAGE. Subsequently, the proteins are transferred to a nylon membrane by electroblotting and the membrane is heated under vacuum to immobilize the proteins. Next, the membrane  
20 is blocked in a solution of 5% w/v BSA in TBS for 2 hrs. at room temperature. Other blocking agents or a non-magnetic colloids or both may be added.

The ferrofluid-labeled antibody probes are then applied to the protein-bound support in 5ml of TBS having an appropriate dilution of the antibody (generally 1:50 - 1:500). The blotting is allowed to occur for 1-3 hrs. at room temperature on a shaker.  
25 The support is then washed in TBS, air dried, and passed before a GMR sensor to detect the magnetic signal present on the support. As a positive control, purified EGF (Sigma) is separated on the SDS/PAGE gel. After the magnetic signal present on the support have been determined, the probes are stripped according to conventional techniques, and the blot is reprobed with the anti-bovine IgG ferrofluid-labeled antibody (control).  
30 Alternatively, total submaxillary gland proteins is separated on a duplicate SDS/PAGE gel, transferred to a membrane, and probed with the ferrofluid-labeled control antibody.

These results demonstrate that the detection of a specific protein in a population of heterogeneous proteins using a ferrofluid-labeled antibody specific for the target protein can be accomplished.

#### EXAMPLE 10

5           In this example, the invention provide methods and systems to identify an antibody-target protein complex by labeling the complex with a ferrofluid and detecting its presence and location using a GMR sensor. Similar to the example above, the probes in this example are the anti-EGF antibody (experimental) and the anti-bovine IgG antibody (control). The target protein is EGF. As in Example 9, total protein from the submaxillary glands from mice (5-20 :g per well) is isolated and separated on a 15% SDS/PAGE. As a positive control, purified EGF (Sigma) is also separated on the gel. The separated proteins in the gel are transferred by electroblotting to a membrane and are immobilized to the support by heating under vacuum. The support is then blocked for 2 hrs. at room temperature on a shaker in a solution of 5% BSA w/v TBS (Sigma).  
10           Other blocking agents or a non-magnetic colloids or both may be added.  
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          After blocking, the support is suspended in 3ml of TBS and an appropriate dilution (generally 1:50 - 1:500) of unlabeled anti-EGF antibody is provided. Binding is allowed to occur for 1-2 hrs., at room temperature on a shaker, and, subsequently, the support is washed in TBS and air-dried. Next, 3 ml of a ferrofluid solution (1:1 v/v) is added to the support and is allowed to interact with the proteins on the support for 5-10 minutes. After a sufficient time, the unbound ferrofluid is removed from the support by successive washes of TBS. The support bound with ferrofluid is air-dried and is passed before a GMR sensor to detect the magnetic signal present. Because the protein attached to an antibody will generate a greater magnetic signal than unbound protein on the support, a determination of the presence and location of the target protein on the support can be made.  
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          Once the magnetic signal on the support has been measured, the probe is stripped from the support, by conventional techniques, and the support is reprobed with the control antibody. Alternatively, a duplicate SDS/PAGE gel can be run on total proteins isolated from the submaxillary glands from mice and the proteins can be transferred to a membrane and probed with the control antibody. The results from this  
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example will demonstrate that conventional techniques in immunoblotting can be readily adapted for use with embodiments of the present invention.

## EXAMPLE 11

5 The invention also provides methods and systems to detect several target proteins on a support by using multiple ferrofluid-labeled probes. In this example, the invention detects several target proteins in the same reaction by using multiple ferrofluid-labeled antibody probes. The probes in this example include a first antibody, the anti-EGF antibody described above, and a second antibody anti-Golgi 58K protein (Sigma G2404). The anti-golgi 58K protein recognizes an epitope on the microtubule-binding peripheral Golgi membrane 58K protein. (Bloom and Brashear *J. Biol. Chem.*, 10 264:16083 (1989)). The first target protein is EGF and the second target protein is the 58K protein. The first antibody is labeled with a first magnetic label (e.g., a first ferrofluid) and the second antibody is labeled with a second magnetic label (e.g., a second ferrofluid) such that the first and the second magnetic labels are different and exhibit different magnetic signals. Labeling is accomplished, as described above. 15

Total protein from mouse submaxillary glands is isolated, according to conventional techniques, and is separated on a 15% SDS/PAGE (5-20 ug per well). As in the example above, after gel electrophoresis, the separated proteins are transferred to a membrane by electroblotting and are immobilized to the support by heating the 20 membrane under vacuum. Subsequently, the membrane is blocked in a solution of 5% w/v BSA and TBS (Sigma) for 2 hrs. at room temperature). Other blocking agents and/or a non-magnetic colloids may be added.

Once blocking is complete, the support is suspended in 3ml of TBS and an appropriate dilution of the magnetically-labeled probes (generally 1:50 - 1:500 for the 25 first probe and 1:5000 for the second probe) is added. Binding is allowed to take place for 1-2 hrs. at room temperature on a shaker and, subsequently, the membrane is washed in TBS and allowed to air dry.

In this embodiment detection is accomplished with a vibrating sample magnetometer or other suitable instrument as described above. Because the first and 30 second probe are labeled with different magnetic labels, having unique magnetic signals, the detection of one or both of the antibody probes can be accomplished in the

same reaction. The results of this example demonstrate that several proteins present in a biological sample disposed on a support can be detected in the same reaction by using different antibody probes having unique magnetic labels.

#### EXAMPLE 12

5           The invention also rapidly detects the presence of a target protein molecule in a solution by using a probe attached to a colored support. In this example, the invention provide methods and systems to rapidly detect a target protein in a solution by using a ferrofluid-labeled probe. Accordingly, an antibody specific for a target biomolecule is attached to a colored support. In one embodiment, the probe antibody is the anti-EGF  
10           antibody, described above. The probe antibody can be coupled to a colored bead by conventional techniques (e.g., a Protein A bead or a Streptavidin bead coupled to a biotinylated anti-mouse IgG antibody). The immobilized probe antibody is then placed in a solution containing total protein isolated from mouse submaxillary glands. Binding is allowed to occur for a period of time and the colored beads are washed in TBS  
15           (Sigma). After washing, the beads are resuspended in TBS and a thin magnet is placed near the side of the vessel. The presence of EGF in the solution is indicated by the aggregation of colored beads near the magnet. These results demonstrate a rapid method to determine the presence of a specific protein in a solution.

#### EXAMPLE 13

20           The invention includes methods and systems that perform competitive binding studies. In this example, the invention provides methods and systems to determine competitive binding of two molecules, each having a unique magnetic label. Competitive binding or competitive inhibition studies are useful for the determination of the avidity of biomolecules which interact with a target biomolecule. In one  
25           embodiment, the target biomolecule is a nucleic acid sequence and the competitive binding of a first nucleic acid sequence (probe 1) which has 100% complementarity to the target biomolecule and a second nucleic acid sequence (probe 2) which has only 80% complementarity to the target biomolecule is studied by labeling probe 1 with a ferrofluid that is different than the ferrofluid used to label probe 2.

30           The target biomolecule and the two ferrofluid-labeled probes are brought in contact with each other in a hybridization solution and hybridization is allowed to take



place for 16 hrs. at a moderately stringent temperature (e.g., 37°C). After hybridization, the support is washed in a suitable wash buffer, air-dried, and the magnetic characteristics of the bound magnetic probes are measured. The presence of the first and/or second magnetic signal on the support, representative of the presence of the first and/or second probe hybridized to the target biomolecule, can be determined over time. By conducting serial hybridizations, for example, and stopping each at various time points, the kinetics of competitive binding can be efficiently evaluated. Because the two magnetic labels attached to the two probes have unique magnetic signals, their presence on the support can be determined in the same reaction.

This embodiment can also be used to study competitive binding of protein ligands, such as antibodies, to a shared epitope on a target biomolecule. As detailed above, the two ligands are magnetically labeled with two different magnetic markers and immunoblotting with the two magnetically-labeled probes is carried out. The presence of the magnetic labels and, thus, the ligands, on the support is evaluated. As above, the detection of one or both of the magnetic labels can be accomplished in the same reaction and the binding kinetics can be studied over time. The use of multiple probes which bind to the same target biomolecule can also be accomplished by using the approaches detailed above so long as each probe has a different magnetic label having a unique magnetic signal. Results from these examples demonstrate that conventional competitive binding assays can be adapted to and enhanced by embodiments of the present invention. More specifically, the use of this embodiment will allow for a dynamic determination of competitive binding in several biochemical interactions including ligand-receptor, antibody-antigen, and nucleic acid hybridization assays.

Preferred embodiments of the present invention have been disclosed. A person of ordinary skill in the art would realize, however, that certain modifications would come within the teachings of this invention, and the following claims should be studied to determine the true scope and content of the invention. In addition, the methods and structures of the present invention can be incorporated in the form of a variety of embodiments, only a few of which are described herein. It will be apparent to the artisan that other embodiments exist that do not depart from the spirit of the invention. Thus,

[illegible][illegible][illegible]

CLAIMS

What is claimed is:

1. A method for assaying molecules in a sample comprising the steps of:
  - providing a sample which contains one or more target molecules or molecular complexes;
  - contacting said target with one or more probes under conditions which permit the formation of a target-probe complex, wherein the probe comprises one or more magnetic labels;
  - subjecting said target-probe complex to an applied magnetic field; and
  - determining one or more magnetic characteristics of said target-probe complex.
2. The method of Claim 1, wherein said target molecule or molecular complex is disposed on a support.
3. The method of Claim 2, wherein said target molecule or molecular complex is disposed on the support on an array.
4. The method of Claim 3, wherein said array comprises an addressable array.
5. The method of Claim 1, wherein said probe is disposed on a support.
6. The method of Claim 5, wherein said probe is disposed on the support on an array.
7. The method of Claim 6, wherein said array is an addressable array.
8. The method of Claim 1, wherein said determining comprises measuring the magnetization of said target-probe complex in response to said applied magnetic field.
9. The method of Claim 1, wherein said determining comprises providing magnetic sensing means that generates a signal in response to said magnetic characteristic.
10. The method of Claim 9, wherein said sensing means comprises a giant magnetoresistive ratio sensor.

11. The method of Claim 9, wherein said determining comprises providing signal processing means that generates readable output from said signal.

12. The method of Claim 1, wherein said determining comprises moving the support or the sensor in relation to the other in one or more directions by transportation means.

13. The method of Claim 1, further comprising subjecting said target-probe complex to one or more of a plurality of applied magnetic fields having different intensities.

14. The method of Claim 1, further comprising subjecting said target-probe complex to one or more of a plurality of applied magnetic fields having different directions.

15. The method of Claim 1, further comprising contacting the target molecule or molecular complex with a non-magnetic colloid.

16. The method of Claim 1, wherein said probe is joined to one or more colored beads, fluorescent beads, or fluorescent cells.

17. The method of Claim 16, further comprising the step of detecting the presence of said target-probe complex by visual, electronic or optical means.

18. A method of isolating one or more biomolecules in a sample comprising the steps of:

- providing a sample containing one or more target biomolecules or molecular complexes;
- contacting the target with one or more probes under conditions which permit the formation of a target-probe complex, wherein the probe comprises one or more magnetic labels; and
- applying a magnetic field to said target-probe complex which isolates said complex according to one or more magnetic characteristics.

19. The method of Claim 18, wherein said magnetic label comprises a ferrofluid.

20. The method of Claim 18, wherein said probe is joined to one or more colored beads, fluorescent beads, or fluorescent cells.

21. The method of Claim 20, further comprising the step of detecting the presence of said target-probe complex by visual, electronic or optical means.

22. A method of identifying the presence of magnetically labeled biomolecules in a sample comprising the steps of:

- providing a sample containing one or more biomolecules;
- contacting the target biomolecule with a magnetic label under conditions which permit the formation of a biomolecule-label complex;
- subjecting said sample to an applied magnetic field; and
- detecting a characteristic response of said sample to an applied magnetic field.

23. The method of Claim 22, wherein said characteristic response is defined at least in part by an induced magnetization of said sample.

24. The method of Claim 22, wherein said characteristic response is defined at least in part by an induced orientation change of said magnetically labeled biomolecules.

25. A method for nucleic acid hybridization comprising the steps of:

- providing one or more single stranded target nucleic acids disposed on a support;
- contacting the single stranded target nucleic acid with one or more nucleic acid probes under conditions which promote the formation of a nucleic acid hybrid, wherein the nucleic acid probe comprises a nucleotide sequence complementary to the single stranded target nucleic acid;
- contacting the nucleic acid hybrid with a ferrofluid whereby the ferrofluid binds to the single stranded target nucleic acid and the nucleic acid hybrid; and
- determining a difference in magnetic signal between the single stranded target nucleic acid and the nucleic acid hybrid.

26. The method of Claim 25, further comprising contacting the single stranded target nucleic acid with a non-magnetic colloid.

27. A method for nucleic acid hybridization comprising the steps of:

- providing a sample that may include a first or a second target nucleic acid disposed on a support;
- contacting the sample with a first nucleic acid probe under conditions which permit the formation of a first nucleic acid hybrid, wherein the first nucleic acid probe comprises a nucleic acid sequence complementary to the first target nucleic acid and a first label having a first magnetic characteristic;
- contacting the sample with a second nucleic acid probe under conditions which permit the formation of a second nucleic acid hybrid, wherein the second nucleic acid probe comprises a nucleic acid sequence complementary to the second target nucleic acid and a second label having a second magnetic characteristic; and
- identifying the presence of the first nucleic acid hybrid by detecting the first magnetic characteristic.

28. The method of Claim 27, further comprising identifying the presence of the second nucleic acid hybrid by detecting the second magnetic characteristic.

29. The method of Claim 28, wherein the presence of the first and second nucleic acid hybrids is identified by detecting one or more magnetic characteristics produced by the combined first magnetic characteristic and the second magnetic characteristic.

30. The method of Claim 27, further comprising contacting the sample with a non-magnetic colloid.

31. A method of nucleic acid hybridization comprising the steps of:

- providing one or more single stranded nucleic acid probes joined to a support so as to form a probe-support complex;
  - providing one or more single stranded target nucleic acids wherein the single stranded target nucleic acid complements the probe nucleic acid and the single stranded target nucleic acid is joined to a magnetic label;
  - contacting the probe-support complex with the single stranded target nucleic acid under conditions which permit hybridization so as to form a target-probe-support complex;
  - applying a magnetic field to the target-probe-support complex;
- and
- identifying the presence of the target-probe-support complex by an accumulation of target-probe-support complex induced by the magnetic field.

32. The method of Claim 31, wherein said probe is joined to one or more colored beads, fluorescent beads, or fluorescent cells.

33. The method of Claim 32, further comprising the step of detecting the presence of said target-probe-support complex by visual, electronic or optical means.

34. A method of increasing binding efficiency comprising the steps of:

- providing one or more target molecules or molecular complexes;
- providing one or more probes, wherein the probe comprises one or more magnetic labels; and
- guiding the probe toward the target with an applied magnetic field.

35. The method of Claim 34, further comprising guiding unbound labeled probe away from the target with an applied electric field.

36. A method for identifying the presence of a protein comprising the steps of:

- providing a target protein disposed on a support;
- contacting the disposed protein with a probe under conditions which promote the formation of a target protein-probe complex, wherein the probe comprises a domain which binds to the target protein and a magnetic label;
- subjecting said target protein-probe complex to an applied magnetic field; and
- determining a magnetic characteristic of said target protein-probe complex.

37. The method of Claim 36, wherein said determining comprises measuring the magnetization of said target protein-probe in response to said applied magnetic field.

38. The method of Claim 36, further comprising contacting the target protein with a non-magnetic colloid.

39. The method of Claim 36, wherein the probe is an antibody or fragment thereof.

40. A method for identifying the presence of a protein comprising:

- providing a target protein disposed on a support;
- contacting the target protein with a probe under conditions which promote the formation of a target protein-probe complex, wherein the probe comprises a domain which binds to the target protein;
- contacting the target protein-probe complex with a ferrofluid whereby the ferrofluid binds to the target protein and the target protein-probe complex; and
- detecting a difference in magnetic signal between the target protein and the target protein-probe complex.

41. The method of Claim 40, further comprising contacting the target protein with a non-magnetic colloid.



42. A method for identifying the presence of a protein comprising the steps of:

- providing a sample that may include a first or a second target protein disposed on a support;
- contacting the sample with a first probe under conditions which permit the formation of a first target protein-probe complex, wherein the first probe comprises a domain which binds to the target protein and a magnetic label having a first magnetic characteristic;
- contacting the sample with a second probe under conditions which permit the formation of a second target protein-probe complex, wherein the second probe comprises a domain which binds to the first target protein and a magnetic label having a second magnetic characteristic;
- identifying the presence of the first target protein-probe complex by detecting the first magnetic characteristic.

43. The method of Claim 42, further comprising identifying the presence of the second target protein-probe complex by detecting the second magnetic characteristic.

44. A method of identifying the presence of a protein comprising the steps of:

- providing a probe joined to a support so as to form a probe-support complex;
  - providing a target protein, wherein the target protein interacts with the probe and the target protein is joined to a magnetic label;
  - contacting the probe-support complex with the target protein under conditions which permit an interaction so as to form a target-probe-support complex;
  - applying a magnetic field to the target-probe-support complex;
- and
- identifying the presence of the target-probe-support complex by an accumulation of target-probe-support complex induced by the magnetic field.

45. A method of competitive binding analysis comprising the steps of:  
providing a target biomolecule;

- contacting the target biomolecule with a first probe molecule comprising a domain which binds to the target biomolecule and a first magnetic label having a first magnetic characteristic;
- contacting the target biomolecule with a second probe molecule comprising a domain which binds to the target biomolecule at the same site as the first probe and a second magnetic label having a second magnetic characteristic;
- identifying the presence of the first probe on the target biomolecule by detecting the first magnetic characteristic; and
- identifying the presence of the second probe on the target biomolecule by detecting the second magnetic characteristic.

46. The method of Claim 45, further comprising the step of detecting a change in the relative preponderance of the first magnetic characteristic and the second magnetic characteristic over time.

47. The method of Claim 45, further comprising contacting the target biomolecule with a non-magnetic colloid which binds to the target biomolecule.

48. A method for identifying an interaction of a probe biomolecule with a target biomolecule comprising the steps of:

- providing a target biomolecule in a fluid;
- providing a probe biomolecule which interacts with the target biomolecule, wherein said probe biomolecule comprises a magnetic label;
- contacting the target biomolecule with the probe biomolecule under conditions which permit the formation of a target biomolecule-probe complex; and
- comparing the magnetic swing of the probe biomolecule in an applied magnetic field with the magnetic swing of the target biomolecule-probe complex in an applied magnetic field.

49. A method of enhancing the binding of a probe biomolecule to a target biomolecule comprising the steps of:

- providing a target biomolecule disposed on a support;
- providing a probe biomolecule comprising a magnetic label;
- contacting the target biomolecule with the probe biomolecule,

wherein the probe biomolecule interacts with the target biomolecule; and

- applying a magnetic field to the target biomolecule and the probe biomolecule such that the probe biomolecule is induced to move toward the disposed target biomolecule.

50. The method of Claim 49, wherein the magnetic field is applied in a pulsing fashion.

51. The method of Claim 50, further comprising the step of applying an electrical field and the magnetic field to the target biomolecule and the probe biomolecule such that the concentration of the probe biomolecule at a site near the disposed target biomolecule is enhanced.

52. The method of Claim 51 wherein the electric field is administered in a pulsing fashion.

53. The method of Claim 49, further comprising the step of applying an electrical field and the magnetic field to the target biomolecule and the probe biomolecule such that the concentration of the probe biomolecule at a site near the disposed target biomolecule is enhanced and by the coordination of the electrical field and the magnetic field the unbound and unlabeled probe is removed.

54. A method of reducing non-specific binding of a probe biomolecule during a binding assay comprising the steps of:

- providing one or more target molecules or molecular complexes disposed on a support;
- contacting the target with one or more probes under conditions which permit the formation of a target-probe complex, wherein the probe comprises one or more magnetic labels;
- subjecting said target-probe complex to an applied magnetic field; and
- administering an electrical field and a magnetic field to the support and the probesuch that non-specifically bound probe is separated from the target biomolecule.

55. The method of Claim 54, further comprising administering a pulsing electrical field and a pulsing magnetic field to the support and the probe biomolecule.

56. A method of nucleic acid separation comprising the steps of:

- providing a sample having one or more polynucleotides in a solution;
- contacting the sample with a ferrofluid whereby the ferrofluid binds to one or more polynucleotides;
- administering a magnetic field to the sample such that one or more of nucleic acids are induced to separate according to their mass.

57. An apparatus for detecting a target molecular species, comprising:

- a magnetoresistive or magnetostrictive magnetic field sensor having attached thereto binding molecules which selectively bind the target molecular species;
- magnetizable label particles bearing binding molecules which:
  - (1) selectively bind the target molecular species or, in the alternative,
  - (2) selectively bind the sensor bound binding molecules, and
    - (i) compete for said selective binding of said sensor bound binding molecules with said selective binding of said target molecular species to said sensor bound binding molecules, or
    - (ii) are displaced from said sensor bound binding molecules by said selective binding of said target molecular species to said sensor bound binding molecules,

whereby the label particles attach to the magnetic field sensor as a result of said selective binding;

- magnetizing means for magnetizing the attached label particles;
- and

- detection means for monitoring a magnetoresistive or magnetostrictive response of the magnetic field sensor to a magnetic field produced by one or more magnetized label particles attached to said magnetic field sensor, whereby the concentration of the target molecule is determined.

58. An apparatus according to Claim 57, wherein the magnetizable label comprises one or more materials selected from the group consisting of paramagnetic, superparamagnetic, ferromagnetic, and ferrimagnetic materials.

59. An apparatus according to Claim 58, wherein the magnetizable label comprises superparamagnetic, iron oxide-impregnated polymer beads.

60. An apparatus according to Claim 57, wherein the magnetic field sensor comprises a magnetoresistive material.

61. An apparatus according to Claim 60, wherein the magnetoresistive material is anisotropic magnetoresistive; giant magnetoresistive; or colossal magnetoresistive.

62. An apparatus according to Claim 57, wherein the magnetic field sensor is magnetostrictive.

63. An apparatus according to Claim 57, wherein the magnetic field sensor is a magnetoresistive element made by patterning a magnetoresistive film.

64. An apparatus according to Claim 57, wherein there are a plurality of magnetic field sensors.

65. An apparatus according to Claim 64, wherein each magnetic field sensor detects the presence or absence of a single label particle attached to said magnetic field sensor.

66. An apparatus according to Claim 57, further comprising means to add the target molecules and the magnetizable particles to the sensor.

67. An apparatus according to Claim 57, wherein the magnetizing means is a magnetic field generator and preferably a wire coil, a straight wire, a conductive microfabricated trace, or a permanent magnet.

68. An apparatus according to Claim 57, further comprising means to remove nonspecifically-bound label particles.

69. An apparatus according to Claim 68, wherein the nonspecifically-bound label particles are removed by a means for applying a magnetic force to the particles and preferably an electromagnet, an air-cored coil, or a permanent magnet.

70. An apparatus according to Claim 69, wherein the means to apply the magnetic force is the magnetizing means for magnetizing the label particles.

71. An apparatus according to Claim 57, wherein the binding molecules are antibodies; poly- or oligo-nucleotides, i.e. DNA or RNA; proteins; synthetic polypeptides; or chelators.

72. An apparatus according to Claim 57, wherein the target molecules are selected from the group consisting of polynucleic acids, proteins, metal ions, and low molecular weight organic species such as toxins, illicit drugs, and explosives.

73. An apparatus according to Claim 57, wherein the detection means comprises a Wheatstone bridge for monitoring both a magnetic field sensor having closely-attached binding elements and an identical field sensor without binding elements.

74. A method for detecting the presence of a target molecular species in a sample suspected of including said target species, comprising the steps of:

a) providing a detector comprising a magnetoresistive or magnetostrictive magnetic field sensor having binding molecules which selectively bind to the target molecules and which are bound to the sensor;

b) bringing the sample and one or more species of magnetizable label particles into contact with the detector at the same or at different times, said label particles having attached binding molecules that;

(1) selectively bind the target species, or, in the alternative;

(2) selectively bind the sensor-bound binding molecules, and

(i) compete for said selective binding of said sensor-bound binding molecules with said selective binding of said target molecular species to said sensor-bound binding molecules, or

(ii) are displaced from said sensor-bound binding molecules by said selective binding of said target molecular species to said sensor-bound binding molecules,

whereby said label particles attach to the sensor as a result of said selective binding, the number of said label particles attached to said sensor being related to the concentration of the target species;

c) removing unbound label particles;

d) magnetizing the remaining bound label particles; and

e) monitoring a magnetoresistive or magnetostrictive response of said magnetic field detector to a magnetic field produced by one or more magnetized label particle attached to said magnetic field sensor, whereby the concentration of the target molecule is determined.

75. A method according to Claim 74, wherein the magnetizable label comprises one or more materials selected from the group consisting of paramagnetic, superparamagnetic, ferromagnetic, and ferrimagnetic materials.

76. A method according to Claim 75, wherein the magnetizable label comprises superparamagnetic iron oxide-impregnated polymer beads.

77. A method according to Claim 74, wherein the magnetic field sensor comprises a magnetoresistive material.

78. A method according to Claim 77, wherein the magnetoresistive material is anisotropic magnetoresistive; giant magnetoresistive; or colossal magnetoresistive.

79. A method according to Claim 74, wherein the magnetic field sensor is magnetostrictive.

80. A method according to Claim 74, wherein the magnetic field sensor is a magnetoresistive element made by patterning a magnetoresistive film.

81. A method according to Claim 74, wherein there are a plurality of magnetic field sensors.

82. A method according to Claim 81, wherein each magnetic field sensor detects the presence or absence of a single label particle attached to said magnetic field sensor.

83. A method according to Claim 74, wherein the bound label particles in step (d) are magnetized by a magnetic field generator and preferably by a wire coil, a straight wire, a conductive microfabricated trace, or a permanent magnet.

84. A method according to Claim 74, wherein the unbound label particles are removed by applying a magnetic force to the particles and preferably by an electromagnet, an air-cored coil, or a permanent magnet.

85. A method according to Claim 84, wherein the magnetic force is applied by the magnet field generator for magnetizing the label particles.

86. A method according to Claim 74, wherein the binding molecules are antibodies; poly- or oligo- nucleotides, i.e. DNA or RNA; proteins; synthetic polypeptides; or chelators.

87. A method according to Claim 74, wherein the target molecules are selected from the group consisting of polynucleic acids, proteins, metal ions, and low molecular weight organic species such as toxins, illicit drugs, and explosives.

88. A method according to Claim 74, wherein the monitoring in step (e) uses a Wheatstone bridge for monitoring both a magnetic field sensor having closely-attached binding elements and an identical field sensor without binding elements.



89. A method according to Claim 74, wherein the contacting in step (b) is done by placing said sample in a solution, applying the solution to the detector, then applying a second solution containing one or more species of magnetizable label particles, wherein said particles have attached label-bound binding molecules with a selective binding response to the target molecules, whereby in the presence of said target molecules said label-bound binding molecules and said sensor-bound binding molecules will sandwich said target molecule, thereby forming a link between said magnetizable label particle and said sensor.

90. An apparatus for sensing a molecule having a magnetic member attached thereto, the magnetic member producing a magnetic field, the apparatus comprising:

- a first magnetoresistive member;
- a first molecular receptor including a first chain of a plurality of nucleotides complementary to a second chain of a plurality of nucleotides of the molecule, the first molecular receptor attached to the first magnetoresistive member so that when the molecule is bound with the first molecular receptor, the magnetic field from the magnetic member influences a magnetization-dependent characteristic of the first magnetoresistive member in accordance with a giant magnetoresistance effect; and
- a readout device coupled to the first magnetoresistive member to produce an electrical signal dependent upon the magnetization-dependent characteristic of the first magnetoresistive member when the molecule is bound with the molecular receptor.

91. The apparatus of Claim 90 wherein the first molecular receptor includes at least one DNA probe, wherein the molecule includes a DNA molecule, and wherein the at least one DNA probe is complementary to the DNA molecule.

92. The apparatus of Claim 90 further comprising a substrate, wherein the readout device includes a transistor integrated with the substrate, and wherein the transistor is coupled to the first magnetoresistive member.

- a second magnetoresistive member; and

94. The apparatus of Claim 93 wherein the second molecular receptor  
es a third chain of a plurality of nucleotides non-complementary to the second

95. The apparatus of Claim 93 further comprising a substrate having the first  
 100. magnetoresistive member and the second magnetoresistive member integrated  
 105. with.

96. A method of sensing a molecule having a magnetic member attached thereto, the method comprising the steps of:

- sensing the magnetization-dependent characteristic of the magnetoresistive member when the molecule is bound with the molecular receptor.

98. The method of Claim 96 wherein the molecular receptor includes a chain  
urality of nucleotides, and wherein the molecule includes a complementary chain  
urality of nucleotides.

99. The method of Claim 98 wherein the molecular receptor includes at least one DNA probe, wherein the molecule includes a DNA molecule, and wherein the at least one DNA probe is complementary to the DNA molecule.

100. The method of Claim 96 wherein the molecule and the molecular receptor include a complementary antibody-antigen specific binding pair.

101. The method of Claim 96 wherein the step of sensing the magnetization-dependent characteristic of the magnetoresistive member includes sensing a magnetization-dependent conductance of the magnetoresistive member.

102. The method of Claim 101 further comprising the steps of:

providing a readout device coupled to the magnetoresistive member; and

producing an electrical signal indicative of the magnetization-dependent conductance of the magnetoresistive member using the readout device.

**HIGH SENSITIVITY BIOMOLECULE DETECTION  
WITH MAGNETIC PARTICLES****ABSTRACT OF THE DISCLOSURE**

The present invention generally relates to the field of biomolecule detection. More specifically, the present invention relates to compositions, methods and systems for the detection and manipulation of biomolecules using magnetic particles.

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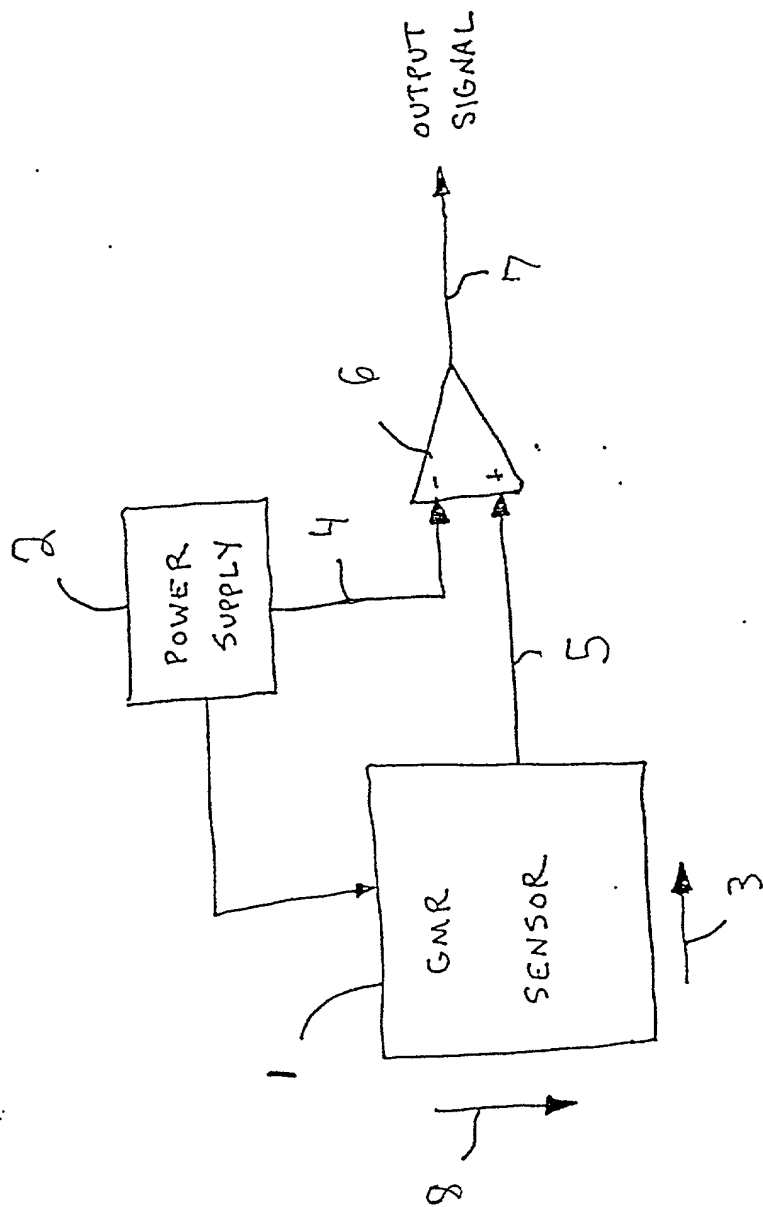


FIG. 1

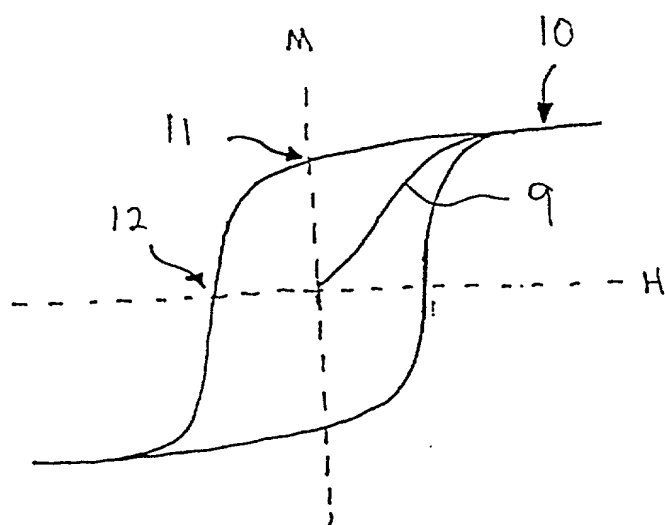


FIG. 2

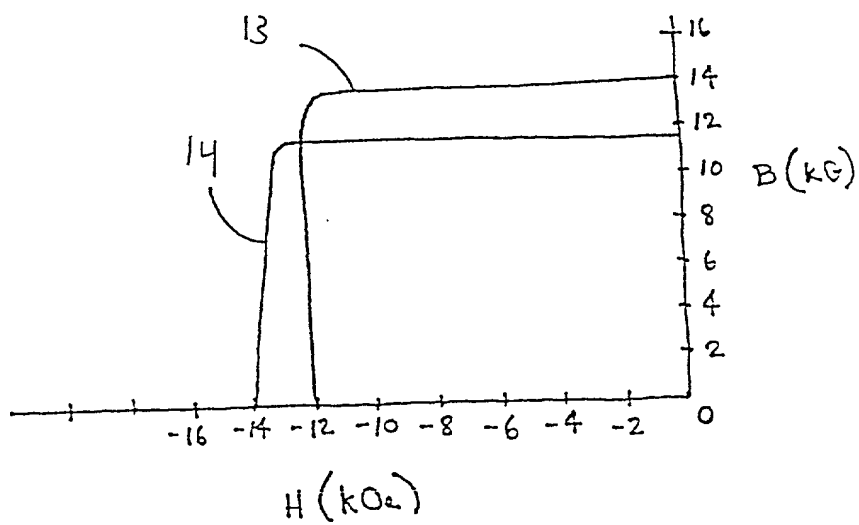


FIG. 3

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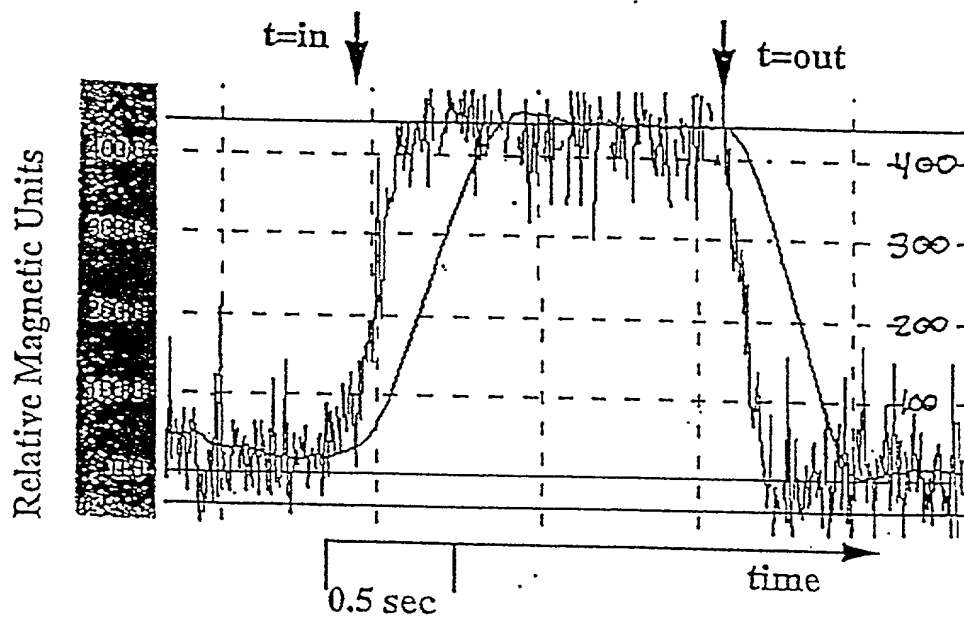


FIG. 4

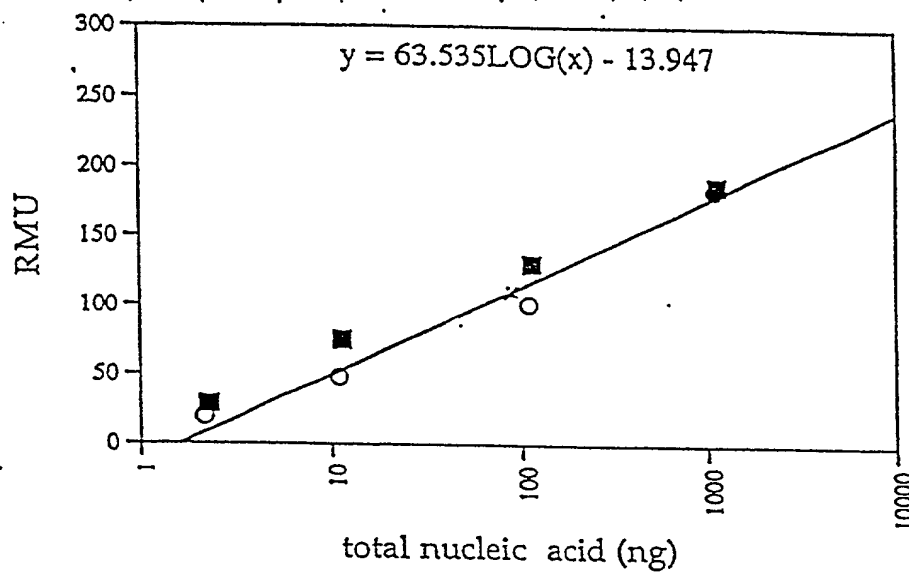


FIG. 5

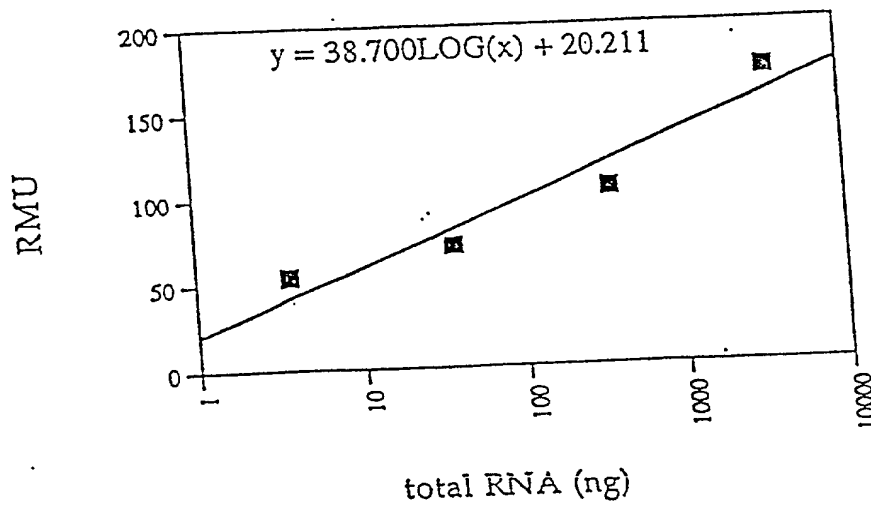


FIG. 6



Practitioner's Docket No. 65446-0053

PATENT

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**COMBINED DECLARATION AND POWER OF ATTORNEY**

(ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL, DIVISIONAL,  
CONTINUATION, OR C-I-P)

---

As a below named inventor, I hereby declare that:

**TYPE OF DECLARATION**

This declaration is for an original application.

**INVENTORSHIP IDENTIFICATION**

My residence, post office address and citizenship are as stated below, next to my name. I believe that I am the original, first and sole inventor (*if only one name is listed below*) or an original, first and joint inventor (*if plural names are listed below*) of the subject matter that is claimed, and for which a patent is sought on the invention entitled:

**TITLE OF INVENTION**

HIGH SENSITIVITY BIOMOLECULE DETECTION WITH MAGNETIC PARTICLES

**SPECIFICATION IDENTIFICATION**

The specification is attached hereto.

**ACKNOWLEDGMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR**

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information, which is material to patentability as defined in 37, Code of Federal Regulations, § 1.56.

**RELATED APPLICATION(S)**

I hereby claim the benefit under Title 35, United States Code §119(e) of United States provisional patent application no. 60/150210, filed August 21, 1999.

Practitioner's Docket No. 65446-0053

**POWER OF ATTORNEY**

I hereby appoint the following practitioner(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

James F. Kamp

Registration Number 41,882

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**DECLARATION**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

**SIGNATURE(S)**

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